Vitamin D, common mental disorders and cognition: insights from genetic and observational epidemiology

A thesis presented for the degree of Doctor of Philosophy

UCL

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Declaration

I, Jane Maddock confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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Jane Maddock

Abstract

The potential relationship between hypovitaminosis D and non-skeletal health outcomes is a growing public health concern. There is suggestion of a relationship between 25-hydroxyvitamin D (25(OH)D) and brain function, with equivocal epidemiological evidence for an association with common mental disorders (CMD) and cognitive function. The aim of the thesis was to investigate the association of 25(OH)D with CMDs and cognitive function in mid-adulthood.

Observational and genetic studies were used to gain better insight into the causal nature of the relationship between 25(OH)D and cognitive function. During observational studies, the association of 25(OH)D with CMDs and cognitive function was assessed in the 1958 British birth cohort (1958BC). A genetic study investigated the potential for a gene-environment interaction (GxE) by *APOE* ε4 on cognitive function using participants from the 1958BC. This GxE study was replicated in an older European cohort. The causal relationship between 25(OH)D and cognitive function was assessed using a Mendelian randomisation (MR) approach in a meta-analyses using participants from nine European cohorts.

Using observational data from 1958BC, there was evidence that both low and high 25(OH)D concentrations were associated with increased risk of CMDs and lower memory function. There was also evidence of a GxE interaction for memory function; where increasing 25(OH)D concentrations may be particularly beneficial for those with *APOE* ε4 genotype. However, results from a MR study provided no evidence for 25(OH)D concentrations acting as a causal factor for cognitive performance in mid- to later-life. Since there was evidence of a non-linear observational association, the MR study may have been underpowered to detect small causal effects at the extremes of the 25(OH)D distribution.

Overall, there is some evidence of a potential non-linear association of 25(OH)D with CMDs and cognitive function. However the causal nature of this relationship requires confirmation from large long-term randomised controlled trials.

Table of contents

ABSTRACT.....3
LIST OF TABLES.....8
LIST OF FIGURES.....10
FREQUENTLY USED ABBREVIATIONS.....12
ACKNOWLEDGEMENTS.....13

CHAPTER 1 INTRODUCTION.....14
1.1 Motivation.....14
1.2 Vitamin D.....15
1.2.1 Discovery of vitamin D.....15
1.2.2 Metabolism of vitamin D.....16
1.2.3 Factors influencing vitamin D status.....20
1.2.4 Vitamin D deficiency and toxicity.....22
1.2.5 Public health importance of vitamin D.....23
1.3 Common mental disorder.....26
1.3.1 Definition of common mental disorder.....26
1.3.2 Prevalence and treatment of common mental disorder.....27
1.3.3 Risk factors for common mental disorders.....29
1.3.4 Public health importance.....33
1.4 Cognition.....33
1.4.1 Definition of cognition.....34
1.4.2 Cognitive disorders in later life.....36
1.4.3 Factors influencing cognition function.....38
1.4.4 Public health importance.....40
1.5 Rationale.....41
1.6 Summary.....42
1.7 Plan of thesis.....43

CHAPTER 2 AIMS AND OBJECTIVES.....45

CHAPTER 3 METHODOLOGICAL APPROACHES.....46
3.1 Introduction.....46
3.2 Observational epidemiology.....46
3.3 Genetic epidemiology.....48

3.3.1 Gene-environment interaction *48*
3.3.2 Mendelian randomisation *52*
3.4 Statistical methods 56
3.4.1 Statistical methods for observational study *56*
3.4.2 Statistical methods for genetic study *61*
3.5 Summary 66

CHAPTER 4 DATA 67
4.1 Introduction 67
4.2 1958 British birth cohort 67
4.2.1 Participants *67*
4.2.2 Exposure *70*
4.2.3 Outcomes *71*
4.2.4 Covariates *79*
4.2.5 Genetic data *81*
4.3 Additional cohorts 87
4.4 Comparison of 1958BC with additional cohorts 100
4.5 Summary 104

CHAPTER 5 OBSERVATIONAL STUDY: ASSOCIATION BETWEEN VITAMIN D AND COMMON MENTAL DISORDER 105
5.1 Introduction 105
5.2 Data and methods 117
5.2.1 Participants *117*
5.2.2 Variables *119*
5.2.3 Statistical analysis *121*
5.3 Results 125
5.3.1 Descriptive characteristics *125*
5.3.2 CMDs and vitamin D-related lifestyles *131*
5.3.3 Cross-sectional association *133*
5.3.4 Prospective association *136*
5.3.5 Additional analysis *139*
5.4 Discussion 143
5.4.1 Conclusion *146*
5.5 Summary 147

CHAPTER 6 OBSERVATIONAL STUDY: ASSOCIATION BETWEEN VITAMIN D AND COGNITIVE FUNCTION 148

6.1 Introduction 148

6.2 Data and methods 168

6.2.1 Participants 168

6.2.2 Variables 170

6.2.3 Statistical analysis 172

6.3 Results 175

6.3.1 Descriptive characteristics 175

6.3.2 Early life factors 180

6.3.3 Vitamin D and cognitive performance 183

6.3.4 Additional analyses 183

6.4 Discussion 188

6.4.1 Conclusion 192

6.5 Summary 193

CHAPTER 7 GENETIC STUDY: GENE-ENVIRONMENT INTERACTION 194

7.1 Introduction 194

7.2 Data and methods 196

7.2.1 Participants 197

7.2.2 Variables 199

7.2.3 Statistical methods 202

7.3 Results 203

7.3.1 Descriptive characteristics 203

7.3.2 Observational association between 25(OH)D and memory 206

7.3.3 APOE ε4 associations 208

7.3.4 GxE interaction 211

7.3.5 Additional analyses 214

7.4 Discussion 217

7.4.1 Conclusion 220

7.5 Summary 220

CHAPTER 8 GENETIC STUDY: MENDELIAN RANDOMISATION TO ASSESS VITAMIN D AND COGNITIVE FUNCTION 221

8.1 Introduction 221

8.2 Data and methods 222

8.2.1 Participants *222*
8.2.2 Variables *224*
8.2.3 Statistical methods *228*
8.3 Results 231
8.3.1 Descriptive results *231*
8.3.2 Observational association *232*
8.3.3 IV validation *240*
8.3.4 Mendelian randomisation analyses *244*
8.4 Discussion 258
8.5 Summary 261

CHAPTER 9 DISCUSSION 262
9.1 Methodological considerations 263
9.2 Main findings 267
9.3 Implications of findings 268
9.4 Potential areas for future research 270

APPENDIX 1 PUBLISHED PAPERS 271
APPENDIX 2 ADDITIONAL INFORMATION FOR CHAPTER 3 312
APPENDIX 3 ADDITIONAL INFORMATION FOR CHAPTER 5 315
APPENDIX 4 ADDITIONAL INFORMATION FOR CHAPTER 6 334
APPENDIX 5: ADDITIONAL INFORMATION FOR CHAPTER 7 350
APPENDIX 6: ADDITIONAL INFORMATION FOR CHAPTER 8 363
REFERENCES 381

List of Tables

CHAPTER 4

TABLE 4.1: 1958BC SWEEP INFORMATION 69

TABLE 4.2: SYMPTOMS OF CMDS MEASURED BY CIS-R AT 45 YEARS IN 1958BC 73

TABLE 4.3: CMD SYMPTOMS ASSESSED USING CIS-R AT 45 YEARS IN 1958BC 74

TABLE 4.4: FACTORS AFFECTING COGNITIVE TESTS AT 50 YEARS IN 1958BC 78

TABLE 4.5: WORD LISTS FOR COGNITIVE TESTS AT 50 YEARS IN 1958BC 78

TABLE 4.6: *APOE* ALLELIC VARIATION AND WORLDWIDE FREQUENCIES 82

TABLE 4.7: DESCRIPTION OF EDUCATION AND DEPRESSIVE VARIABLES IN ADDITIONAL COHORTS 101

TABLE 4.8: COMPARISON OF 1958BC WITH ADDITIONAL COHORTS 102

TABLE 5.1: SYSTEMATIC REVIEW: VITAMIN D AND CMDS 106

TABLE 5.2: COVARIATES FROM 1958BC FOR STUDY ON 25(OH)D AND CMDS 120

TABLE 5.3: CHARACTERISTICS OF PARTICIPANTS WITH ≥2 SYMPTOMS OF CMDS AT 45 YEARS 129

TABLE 5.4: CMDS AND VITAMIN D-RELATED LIFESTYLE FACTORS AT 45 YEARS 132

TABLE 5.5: ASSOCIATION BETWEEN 25(OH)D AND CMDS AT 45 YEARS 134

TABLE 5.6: ASSOCIATION BETWEEN 25(OH)D AND DEPRESSIVE SYMPTOMS AT 50 YEARS 137

TABLE 5.7: DISTRIBUTION OF DIFFERENT MHI-5 THRESHOLDS AT 50 YEARS IN 1958BC .. 139

TABLE 5.8: ASSOCIATION BETWEEN CMDS AND 25(OH)D AT 45 YEARS 140

TABLE 5.9: ASSOCIATION BETWEEN 25(OH)D AND DEPRESSIVE SYMPTOMS (MHI-5 ≤60 AND ≤75) AT 50 YEARS 141

TABLE 5.10: COMPARISON OF COMPLETE, WEIGHED AND IMPUTED CROSS-SECTIONAL AND PROSPECTIVE RESULTS 142

TABLE 6.1: SYSTEMATIC REVIEW: VITAMIN D AND COGNITIVE FUNCTION 149

TABLE 6.2: COVARIATES FROM 1958BC FOR STUDY ON 25(OH)D AND COGNITIVE FUNCTION 171

TABLE 6.3: DISTRIBUTION OF 25(OH)D BY PARTICIPANT CHARACTERISTICS 176

TABLE 6.4: MEAN PERFORMANCE ON COGNITIVE TESTS BY 25(OH)D STATUS 179

TABLE 6.5: ASSOCIATION BETWEEN EARLIER LIFE FACTORS WITH COGNITIVE PERFORMANCE AT 50 YEARS 182

TABLE 6.6: ASSOCIATION BETWEEN 25(OH) D AT 45 YEARS AND COGNITIVE FUNCTION AT 50 YEARS 184

TABLE 6.7: COMPARISON OF COMPLETE, WEIGHED AND IMPUTED RESULTS FOR 25(OH)D ASSOCIATION WITH IMMEDIATE WORD RECALL 186

TABLE 6.8: ASSOCIATION BETWEEN 25(OH) D AT 45 YEARS AND COGNITIVE FUNCTION AT 50 YEARS, ADJUSTED FOR DIETARY SUPPLEMENTS 187

TABLE 7.1: *APOE* GENOTYPE FREQUENCY 200

TABLE 7.2: CHARACTERISTICS OF ELIGIBLE PARTICIPANTS FROM 1958BC AND PIVUS ... 204

TABLE 7.3: DISTRIBUTION OF *APOE* E4 ALLELES 206

TABLE 7.4: MEAN PERFORMANCE ON INDIVIDUAL MEMORY TESTS 206
TABLE 7.5: ASSOCIATION BETWEEN 25(OH)D AND STANDARDISED MEMORY FUNCTION SCORE 207
TABLE 7.6: ASSOCIATION BETWEEN *APOE* E4 AND MEMORY SCORE 210
TABLE 8.1: ELIGIBLE PARTICIPANTS FOR MR STUDY 223
TABLE 8.2: GENDER AND STUDY SPECIFIC 25(OH)D TERTILES IN EACH COHORT 226
TABLE 8.3: CATEGORISATION OF COGNITIVE TESTS IN EACH COHORT 227
TABLE 8.4: ASSOCIATION BETWEEN AND 25(OH)D AND COGNITIVE FUNCTION 233
TABLE 8.5: ASSOCIATION BETWEEN GENETIC VARIANTS AND 25-HYDROXYVITAMIN D 245
TABLE 8.6: META-ANALYSED ASSOCIATION BETWEEN GENETIC VARIANTS AND COGNITIVE FUNCTION: STRATIFIED BY 25(OH)D TERTILES 254
TABLE 8.7: INSTRUMENTAL VARIABLE RATIO 256
TABLE 8.8: INSTRUMENTAL VARIABLE RATIO: STRATIFIED BY 25(OH)D TERTILES 257

List of Figures

CHAPTER 1

FIGURE 1.1: VITAMIN D METABOLISM 18

FIGURE 1.2: PREVALENCE OF 25(OH)D <25NMOL/L IN THE UK 25

FIGURE 1.3: FACTORS ASSOCIATED WITH MENTAL HEALTH 30

FIGURE 1.4: COGNITIVE FUNCTION THROUGHOUT THE LIFESPAN 35

FIGURE 1.5: CROSS-SECTIONAL AND LONGITUDINAL ESTIMATES OF AGE-RELATES CHANGE IN COGNITIVE FUNCTION 37

FIGURE 3.1: PUBMED SEARCH RESULTS FOR *GENE-ENVIRONMENT INTERACTION* BETWEEN 1990 AND 2012 50

FIGURE 3.2: THEORETICAL GENE-ENVIRONMENT INTERACTION ON A HEALTH-RELATED OUTCOME 51

FIGURE 3.3: COMPARISON OF DESIGN OF MR STUDIES WITH RCT 53

FIGURE 3.4: ASSUMPTIONS OF MR; ILLUSTRATED BY 25(OH)D AND COGNITIVE FUNCTION 55

FIGURE 3.5: IV RATIO ESTIMATION; ILLUSTRATED BY 25(OH)D AND COGNITIVE FUNCTION . 63

FIGURE 4.1: COGNITIVE TESTS CONDUCTED AT 50 YEARS IN 1958BC 77

FIGURE 4.2: GENETIC VARIANTS ON THE VITAMIN D METABOLIC PATHWAY 84

FIGURE 5.1: PARTICIPANT SELECTION FOR STUDY ON 25(OH)D AND CMDS 118

FIGURE 5.2: PROPORTION OF MISSING COVARIATE OBSERVATIONS FOR STUDY ON 25(OH)D AND CMDS 124

FIGURE 5.3: COMORBIDITY OF SYMPTOMS OF CMDS AT 45 YEARS 126

FIGURE 5.4: GEOMETRIC MEAN OF 25(OH)D WITH STANDARD ERROR ACCORDING TO CMDS AT 45 YEARS AND DEPRESSIVE SYMPTOMS AT 50 YEARS 128

FIGURE 5.5: PREDICTED PROBABILITY OF DEPRESSIVE SYMPTOMS ACCORDING TO 25(OH)D CONCENTRATIONS AT 45 YEARS 135

FIGURE 5.6: PREDICTED PROBABILITY OF DEPRESSIVE SYMPTOMS AT 50 YEARS ACCORDING TO 25(OH)D CONCENTRATIONS AT 45 138

FIGURE 6.1: PARTICIPANT SELECTION FOR STUDY ON 25(OH)D AND COGNITIVE FUNCTION 169

FIGURE 6.2: PROPORTION OF MISSING COVARIATE OBSERVATIONS FOR STUDY ON 25(OH)D AND COGNITIVE FUNCTION 174

FIGURE 6.3: ASSOCIATION BETWEEN CHILDHOOD COGNITIVE ABILITY AND EDUCATIONAL ATTAINMENT WITH COGNITIVE PERFORMANCE AT 50 YEARS 181

FIGURE 6.4: PREDICTED IMMEDIATE WORD RECALL AT 50 YEARS ACCORDING TO 25(OH)D CONCENTRATIONS AT 45 YEARS 185

FIGURE 7.1 PARTICIPANT SELECTION FOR GXE STUDY IN 1958BC 198

FIGURE 7.2: PERCENTAGE OF PARTICIPANTS WITH <25NMOL/L AND <50NMOL/L 25(OH)D 205

FIGURE 7.3: GEOMETRIC MEAN OF 25(OH)D ACCORDING TO NUMBER OF *APOE* E4 ALLELES 209

FIGURE 7.4. GENE-ENVIRONMENT INTERACTION IN 1958BC 212

FIGURE 7.5: GENE-ENVIRONMENT INTERACTION IN PIVUS 213

FIGURE 7.6: GENE-ENVIRONMENT INTERACTION IN 1958BC, USING RECESSIVE MODEL .. 215
FIGURE 7.7: GENE-ENVIRONMENT INTERACTION IN PIVUS, USING RECESSIVE MODEL..... 216
FIGURE 8.1: ASSOCIATION BETWEEN 25(OH)D AND GLOBAL COGNITIVE FUNCTION..... 235
FIGURE 8.2: ASSOCIATION BETWEEN 25(OH)D AND MEMORY FUNCTION 236
FIGURE 8.3: NON-LINEAR ASSOCIATION BETWEEN 25(OH)D AND COGNITIVE FUNCTION .. 237
FIGURE 8.4: ASSOCIATION OF 25(OH)D WITH GLOBAL COGNITION: STRATIFIED BY 25(OH)D TERTILES 238
FIGURE 8.5: ASSOCIATION OF 25(OH)D WITH MEMORY FUNCTION: STRATIFIED BY 25(OH)D TERTILES 239
FIGURE 8.6: ASSOCIATION OF EFFECT ALLELES WITH 25(OH)D 241
FIGURE 8.7: ASSOCIATION BETWEEN GENETIC VARIANTS AND 25(OH)D, ADJUSTED FOR LIFESTYLE FACTORS 242
FIGURE 8.8: ASSOCIATION BETWEEN GENETIC VARIANTS AND POTENTIAL CONFOUNDERS 243
FIGURE 8.9: ASSOCIATION BETWEEN *DHCR7* AND 25(OH)D 246
FIGURE 8.10: ASSOCIATION BETWEEN *CYP2R1* AND 25(OH)D 247
FIGURE 8.11: ASSOCIATION BETWEEN SYNTHESIS SCORE AND 25(OH)D 248
FIGURE 8.12: ASSOCIATION BETWEEN *DHCR7* AND COGNITIVE FUNCTION 251
FIGURE 8.13: ASSOCIATION BETWEEN *CYP2R1* AND COGNITIVE FUNCTION 252
FIGURE 8.14: ASSOCIATION BETWEEN SYNTHESIS SCORE AND COGNITIVE FUNCTION..... 253

Frequently used Abbreviations

$1,25(OH)_2D$	1,25-dihydroxyvitamin D
1958BC	1958 British birth cohort
25(OH)D	25-hydroxyvitamin D
AD	Alzheimer's Disease
APOE	Apolipoprotein E
ASPS	Austria Stroke Prevention Survey
BMI	Body Mass Index
CI	Confidence Interval
CIS-R	Clinical Interview Schedule-Revised
CMD	Common Mental Disorder
CYP2R1	Cytochrome P450, Family 2, Subfamily R, Polypeptide 1
DEQAS	Vitamin D External Quality Assessment
DHCR7	7-Dehydrocholesterol Reductase
DNA	Deoxyribonucleic acid
ELSA	English Longitudinal Study of Ageing
ESTHER	Epidemiologische Studie zu Chancen der Verhütung, Früherkennung und optimierten Therapie chronischer Erkrankungen in der älteren Bevölkerung
GP	General Practitioner
GWAS	Genome-Wide Association Studies
GxE	Gene-environment interaction
HBCS	Helsinki Birth Cohort Study
HWE	Hardy-Weinberg Equilibrium
IOM	Institute of Medicine
IU	International Units
IV	Instrumental Variable
ln	Natural logarithmic transformation
MAF	Minor Allele Frequency
MHI	Mental Health Inventory
MMSE	Mini-Mental State Examination;
MR	Mendelian Randomisation
NCDS	National Child Development Study
OR	Odds Ratio
PC	Personal Computer
PIVUS	The Prospective Investigation of the Vasculature in Uppsala Seniors
RCT	Randomised Controlled Trials
RDA	Recommended Dietary Allowance
SD	Standard Deviation
SE	Standard Error
SEP	Socioeconomic Position
SNP	Single Nucleotide Polymorphism
TV	Television
UK	United Kingdom
ULSAM	Uppsala Longitudinal Study of Adult Men
VDBP	Vitamin D Binding Protein
VDR	Vitamin D Receptor
WHII	Whitehall II

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Chapter 1 Introduction

1.1 Motivation

Promising investigations into the potential effect of modifiable exposures on mental health and cognitive function throughout the life-course has motivated work for this thesis: *Vitamin D, common mental disorders and cognition: Insights from observational and genetic epidemiology*. The prospect of a true casual effect between vitamin D status and these outcomes could have long term implications by providing an easily modifiable, safe and cheap way to improve human well-being.

There has been a significant move towards promoting mental health and campaigning to reduce stigma and discrimination associated with mental ill-health. In 2013, member states participating in the 66th World Health Assembly adopted the World Health Organisation's (WHO) comprehensive Mental Health Action Plan[1] which emphasises the importance of mental health and specifies targets to be reached by 2020. Further efforts to promote mental health include the establishment of World Mental Health Day in 1992 by the World Federation for Mental Health[2] and Mental Health Awareness week in 2002 by the UK organisation, Mental Health Foundation. Despite these endeavours, mental disorders remain both poorly diagnosed and poorly treated (1). Common mental disorders (CMD), such as depression and anxiety affect a large percentage of the adult UK population (9). Research into the mechanisms and potential risk factors for CMDs could lead to significant improvements in the understanding of the disorder, the quality of life of individuals and may benefit the wider society.

The other topic explored involves cognitive function. Generally, an individual's cognitive ability develops from birth, stabilises in adulthood and declines with increasing age. The prediction of an increasing global population and concurrent shift towards an older demographic highlights the need for the examination of factors related to healthy ageing (10). Through the identification of modifiable exposures that could either maximise cognitive function or delay

[1] http://www.who.int/mental_health/en/index.html
[2] http://www.wfmh.com/index.html

the onset of cognitive decline, the quality of life of older adults could be improved and consequent economic and social benefits could ensue.

Further details on mental health and cognitive function including definition, risk factors and public health importance are given later in this chapter. Also presented is the biological plausibility of vitamin D playing a causal role in mental health and cognitive function. However, it is important first to provide an overview of vitamin D; its discovery, how it is metabolised, factors that can influence vitamin D status and the vitamin D concentration thresholds used to define deficiency and toxicity.

1.2 Vitamin D

1.2.1 Discovery of vitamin D

In the early 19th century, an adequate diet was considered to consist of appropriate amounts of protein (12%), minerals (5%), fat (10-30%) and carbohydrate (remaining %) (11). This view of adequacy began to change with the finding that consumption of certain foods prevented disease for example, consumption of lime to prevent scurvy (11). The realisation that food contained another vital factor relevant for health led to a rapid growth in experimental models. E.V. McCollum and his team are credited as one of the first to identify these vital amines or vitamins with the discovery of vitamin A and the B vitamins (12).

Unravelling the cause of the paediatric bone disease, rickets, led to the discovery of vitamin D (11). At the end of the 19th century, rickets, also known as the English disease, was at epidemic proportions. Rickets was found to affect children living in sunlight-deprived industrial cities as opposed to those living in rural areas or in sunnier climates (13). Sir Edward Mellanby began to explore diet-related explanations for rickets. He found that children with rickets could be treated with cod liver oil. Subsequent work conducted by Mellanby and McCollum identified the antirachitic substance in cod liver oil as vitamin D, which became known as an essential nutrient (11).

During the same period, Mellanby discovered that rickets could also be treated by exposure to sunlight or mercury lamps (11). Furthermore, it was found that food irradiated with ultraviolet B (UVB) can be antirachitic (13). The link between sunlight and rickets coupled with extensive research carried out in the 1970s contributed to the understanding of the dual sources of vitamin D, helping to clarify that the nutrient is in fact a hormone precursor that can be produced in the body rather than a vitamin (13).

Research into the health effects of vitamin D continues to date. Some remaining challenges in vitamin D epidemiology include determining causative relationships with non-skeletal diseases/disorders and establishing effective thresholds for identifying deficiency and population groups at risk.

1.2.2 Metabolism of vitamin D

Since the first discovery of vitamin D, there has been extensive research into the identification and description of the compound and its metabolic pathway. The term vitamin D refers to a group secosteroids i.e. a molecule similar in structure to a steroid but with a broken ring (the 9,10 carbon-carbon bond of ring B is broken in vitamin D) (14). Humans obtain vitamin D mainly through synthesis in the skin following exposure to sunlight and to a lesser extent through dietary sources (15). There are two main forms of the vitamin D nutrient, vitamin D3 and vitamin D2 (**Figure 1.1**).

Vitamin D3 (cholecalciferol), is derived from 7-dehydrocholesterol in the skin following exposure to UVB radiation of wavelengths 290-315nm (16). Vitamin D3 can also be obtained from a limited number of dietary sources such as, oily fish like, salmon, tuna, sardines and mackerel, egg yolk and fortified foods. Vitamin D2 (ergocalciferol) is produced by the UV irradiation of ergosterol from yeast and can occur naturally in some types of mushrooms for example, shiitake (17). Both nutrient forms can also be obtained in supplement form.

Vitamin D3 and D2 both result in an increase of an individual's nutritional status and they follow the same metabolic pathway. However, small structural differences in the side chains of vitamin D3 and D2 (**Figure 1.1**) inspired further

research into their comparative effectiveness. A recent review has demonstrated that despite some evidence implying that vitamin D3 is more effective in raising and maintaining an individual's nutritional status than D2, this does not occur across all studies (18). For the purposes of the thesis, the term, *vitamin D* will be used to represent D2 or D3 unless specified.

The metabolism of vitamin D is illustrated in **Figure 1.1**. Following skin exposure to UVB radiation, 7-dehydrocholesterol, present in cells of the dermis and epidermis, is converted to pre-vitamin D3 and subsequently to vitamin D3. Excessive sunlight exposure degrades pre-vitamin D3 into inactive substances (17). Due to its lipophilic nature, vitamin D requires a protein carrier for transport in circulation and can potentially be taken up by adipose tissue via lipoprotein lipase (19). Dietary sources of vitamin D are incorporated into chylomicrons (which are involved in the transport of dietary lipids) when they enter the circulation.

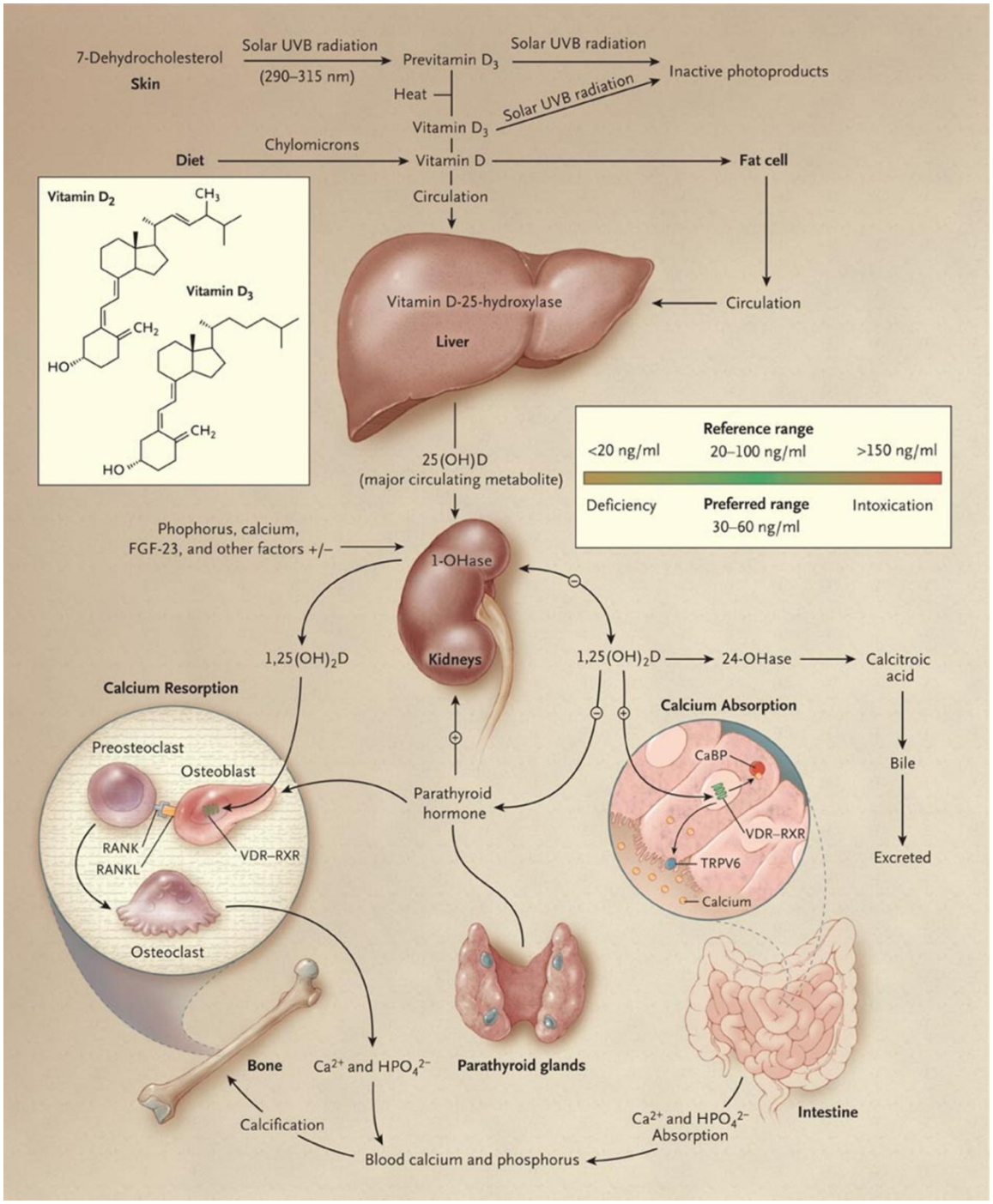

Figure 1.1: Vitamin D metabolism

Taken from Holick, 2007(17)

Once in circulation, vitamin D binds to the vitamin D-binding protein (VDBP) and is transported mainly to the liver where it undergoes its first hydroxylation. Here, vitamin D is converted to 25-hydroxyvitamin D (25(OH)D) by the enzyme 25-hydroxylase. The conversion of vitamin D to 25(OH)D is dependent on vitamin D intake and 25(OH)D concentrations via a negative feedback system (20, 21). 25(OH)D is the major circulating form of vitamin D that is used to determine vitamin D status. This is a good measure of vitamin D nutritional status as it takes into account sun exposure and dietary sources and has an half-life of approximately 15 days (19, 20).

In order for the metabolite to become biologically active, 25(OH)D travels to the kidney and some other organs where it is hydroxylated by the enzyme 25(OH)D-1α-hydroxylase. This hydroxylation produces the biologically active hormone, 1,25-dihydroxyvitamin ($1,25(OH)_2D$, also known as calcitriol). The production of $1,25(OH)_2D$ is tightly regulated and has a serum half-life of 10 to 20 hours (19). Renal regulation of $1,25(OH)_2D$ comprises many factors and is involved in the maintenance of calcium homeostasis. Increased serum phosphorous, calcium and fibroblast growth factor 23 inhibit the production of $1,25(OH)_2D$ while parathyroid hormone (PTH) enhances production (17). PTH is secreted when serum calcium levels are low. This increase in PTH triggers production of $1,25(OH)_2D$ which enables calcium resorption in the kidney. Additionally, when serum calcium levels are low, $1,25(OH)_2D$ increases absorption of calcium in the small intestine and encourages osteoblasts to mobilise calcium and phosphate from the bone to maintain normocalcemia (22).

The role of $1,25(OH)_2D$ in calcium homeostasis and bone health is well established. However, the far-reaching biological effects of $1,25(OH)_2D$ are only beginning to be understood. The diverse biological actions of the hormone $1,25(OH)_2D$ can occur following binding with nuclear vitamin D receptors (VDR). The VDR is a transcription factor i.e. a protein that binds to specific DNA sequences that can control gene expression (23). The VDR can recognise a specific DNA sequence, in this case, the vitamin D response element (VDRE) thereby targeting distinctive genes. Once $1,25(OH)_2D$ binds to the VDR, a heterodimer structure between VDR and a retinoid-X receptor (RXR), which is part of the family of nuclear receptors, is formed. This VDR/RXR structure will

then bind to VDRE in the regulatory region of a vitamin D target gene (24). This process will trigger the expression of the target gene which facilitates the formation of proteins with tissue-specific functions.

Catabolism of excess 25(OH)D and 1,25$(OH)_2$D is mediated by the enzyme 24-hydroxylase where they are converted to water-soluble inactive substance calcitroic acid, and excreted in the bile (17, 20).

1.2.3 Factors influencing vitamin D status

There are a variety of factors from environmental to genetic that can influence an individual's vitamin D status.

As mentioned, one of the main sources of vitamin D is sunlight. Therefore, it is no surprise that factors affecting UVB exposure influence vitamin D status. One element that affects UVB radiation is the solar zenith angle (SZA). The SZA is the angle at which the sun penetrates the earth's atmosphere (21). A small SZA will result in increased UV radiation. Latitude, time of day and seasonality all effect the SZA (25). As expected, in the northern hemisphere, UVB radiation is most effective at mid-day in the summer (25). Thus, in the UK, 25(OH)D levels are lowest during late winter and highest during late summer (26). Furthermore, it has been suggested that the SZA is smaller in latitudes below approximately 350˚ north (27). Consequently, cutaneous production of vitamin D has the potential to be produced all year round in these areas for example, Miami, Florida (250) and Abu Dhabi (240). It was also noted that above latitudes of 350˚ north for example, areas of Boston (420) and Norway (610), UVB radiation is insufficient to stimulate vitamin D synthesis during the winter months (27). Pollution and heavy cloud cover can diminish the number of UVB rays that reach the earth's surface thereby reducing the skin's ability to produce vitamin D (25).

Individual determinates of vitamin D status range from lifestyle factors, ageing, and ethnicity amongst others. As a person ages, skin thickness decreases (25). This results in a reduction of 7-dehydrocholesterol in the epidermis thereby reducing the ability to produce pre-vitamin D (28). Additionally, application of

sunscreen to the skin blocks UVB rays, preventing the synthesis of vitamin D (29). Colour of the skin also plays a role in determining vitamin D status. Melanin is a substance that acts as a natural sunscreen and gives skin its pigmentation (25). Studies have shown that individuals with darker skin and more melanin produce less vitamin D compared with individuals with lighter skin (30).

Obesity has been associated with lower vitamin D status (31, 32). A recent bi-directional Mendelian randomisation study suggested that a higher body mass index (BMI) leads to reduced 25(OH)D concentrations while the effect of lower 25(OH)D concentrations on higher BMI is likely to be small (33). This relationship may be a result of adipose sequestration of vitamin D from circulation, resulting in a reduction of circulating 25(OH)D (34).

Factors such as working conditions and cultural practices can have an effect on vitamin D status. For example, those working in outdoor activities will have greater exposure to sunlight compared to those working indoors (35). Use of clothing to cover the skin can limit the amount of skin available for vitamin D production. For instance, a study conducted in the Lebanon, found that the majority of women who wore veils had 25(OH)D concentrations of less than 12.5nmol/l (36). Dietary factors such as consumption of oily fish and supplements can also influence an individual's vitamin D status (26).

A genetic influence on vitamin D status has been shown. Study estimates of the heritability of vitamin D status range from 29% (Framingham Offspring Study (37)), 43% (Twins UK study (38)) to 80% (German Asthma Study (39)). The discovery of genetic variants associated with the metabolism of vitamin D has escalated in the past several years. These will be discussed further in **Chapter 4**. There is also a hypothesised association between 25(OH)D and Apolipoprotein ε4 alleles (40, 41) which is detailed in **Chapter 7**.

The environmental and individual factors discussed in this section may act independently or interact to initiate or maintain deficient, optimal or toxic concentrations of 25(OH)D.

1.2.4 Vitamin D deficiency and toxicity

The classical characteristics of severe vitamin D deficiency are stunted growth, defective bone mineralisation and muscle weakness. These can manifest as rickets (in children) and osteomalacia (in adults) (42). Deficiency symptoms are the result of the actions of $1,25(OH)_2D$ to maintain calcium homoeostasis (detailed in ***section 1.2.2***). When $1,25(OH)_2D$ is severely reduced, calcium absorption from the intestine is decreased. The resulting hypocalcaemia can trigger calcium mobilisation from bones and result in adverse skeletal, muscular and dental outcomes.

Toxicity symptoms include hypercalcaemia and calcification of soft tissue (19). Toxicity does not occur through intake from sunlight, but can occur via excessive dietary intake or supplement sources of vitamin D. The mechanism of vitamin D toxicity remains elusive. However, it has been suggested that excessive vitamin D intake increases plasma concentrations of 25(OH)D which exceed the VDBP capacity. Therefore, synthesis to $1,25(OH)_2D$ does not occur, resulting in unregulated, free 25(OH)D entering the cell and directly binding to VDR where it can have a direct effect on gene expression (19). Inappropriately high levels of free $1,25(OH)_2D$ may also result in increased intestinal calcium absorption and bone resorption (43).

Determining the exact threshold of 25(OH)D concentrations needed for health has been heavily debated. One position, taken by the Institute of Medicine (IOM), is to set the threshold based on evidence for bone health. Consensus has been reached that everyone should have 25(OH)D above 25nmol/l (42), with the IOM reporting an increased risk of bone disease below 30nmol/l when calcium intake is adequate (44). The IOM continues to reason that the threshold should be at least 50nmol/l which would be sufficient to maintain bone health of 97.5% of the population (44).

The second position in this debate states that the threshold should be increased to at least 75-100nmol/l based on new evidence of the association between vitamin D and non-skeletal disease (45). In 2009, the Rank Forum on Vitamin D declared that before recommendations on 25(OH)D thresholds are confirmed,

optimal vitamin D requirements for non-skeletal health outcomes should be established (42).

There is consensus that levels greater than 250nmol/l can lead to hypercalcemia (19, 46). The IOM toxicity threshold is 125-150nmol/l. This was based on evidence that individuals maintain plasma concentrations of 125-150nmol/l following maximal sun exposure and some adverse health outcomes were reported at concentrations below 125nmol/l (44).

With these thresholds in mind, the IOM set the Recommended Dietary Allowance (RDA) based on the maintenance of 25(OH)D concentrations at 50nmol/L (44). RDA, assuming minimum sun exposure, for males and females aged 9-70 years was set as 600 IU/day (15 µg/day) and 800 IU/day (20 µg/day) for those aged >70 years. Tolerable Upper Intake Level (UL) was estimated to be 4,000 IU/day (100 µg/day) for ages >9 years.

Public health efforts to maintain the population's "optimal" vitamin D status prompted fortification of food with vitamin D. In 1940, the Department of Health UK included cod liver oil and dried fortified milk in its Welfare Food Scheme (47). Voluntary fortification of cereals and evaporated milk followed (48). However, reported cases of hypercalcaemia in infants and children led to a sharp reduction in these fortification efforts. The association between excessive vitamin D fortification and hypercalcaemia was refuted in a review conducted in 1967 (49). Currently, in the UK, vitamin D fortification of margarine (7.05-8.82 µg/100g) and infant formula is mandatory (50, 51).

More research to clarify the association between vitamin D and non-skeletal health is required to conclude this debate.

1.2.5 Public health importance of vitamin D

Due to the lack of a universal agreement of the thresholds of 25(OH)D status, prevalence of vitamin D deficiency varies depending on what cut-points are used. Nevertheless, low serum 25(OH)D concentrations are extremely prevalent in the UK and worldwide (17, 42).

It has been estimated that at least 10% of UK adults have 25(OH)D concentrations below 25nmol/l (**Figure 1.2**) (42). 25(OH)D concentrations have been found to be particularly low in both younger and older adults (52) (**Figure 1.2**). Children from ethnic minorities residing in the UK were also at risk of lower 25(OH)D concentrations (53). Results from a longitudinal study conducted in Southampton, UK, showed that maternal vitamin D insufficiency (i.e. 25-50nmol/l 25(OH)D) was common during pregnancy and associated with poor bone health in children (54). Furthermore, the Health for England survey carried out in 2005 observed that the majority of adults aged ≥65 years (57% of women and 49% men) had 25(OH)D concentrations of less than 50nmol/l (55).

Reduced 25(OH)D concentrations have been associated with a myriad of adverse health outcomes including autoimmune, cardiovascular and infectious disease (56). Therefore, low 25(OH)D status constitutes a public health problem which has been largely attributed to modern lifestyle behaviours, characterised by increasing obesity, indoor working and proficient use of suncover (26, 35).

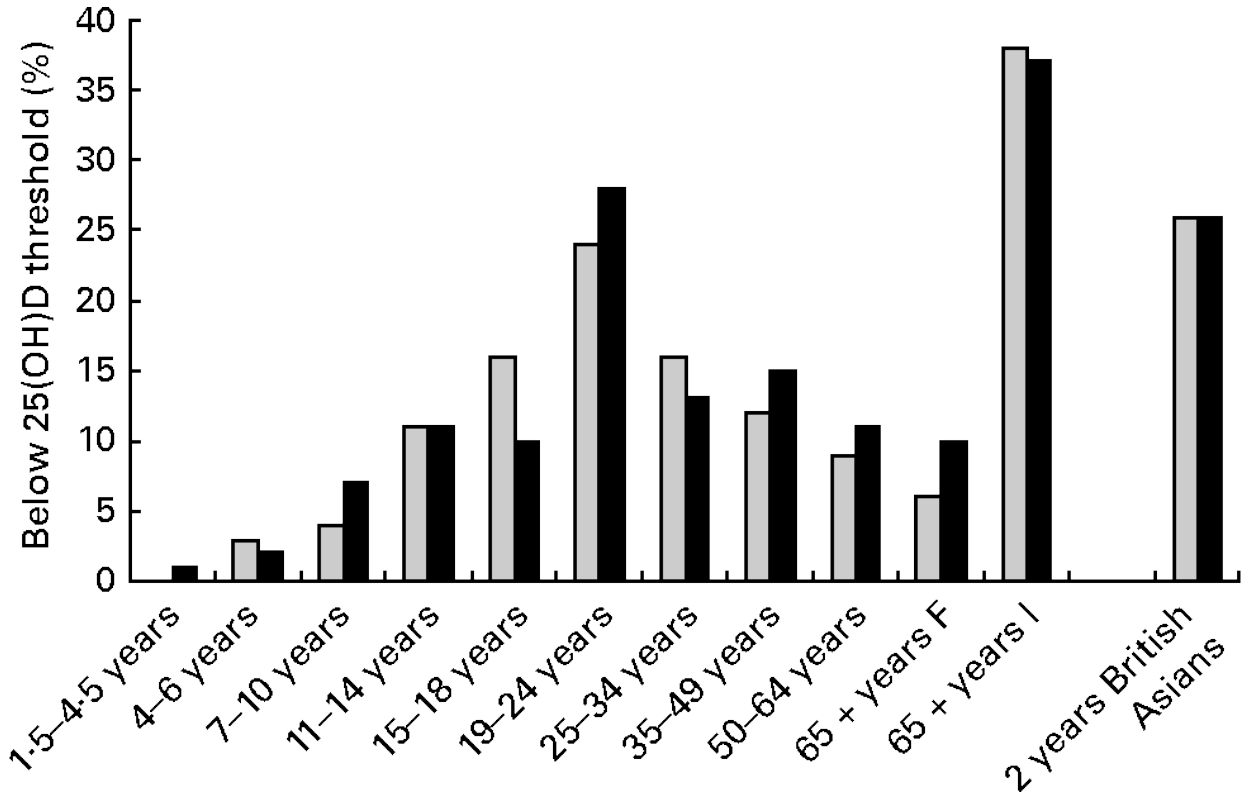

Figure 1.2**:** Prevalence of 25(OH)D <25nmol/l in the UK

British Asians are defined as those of South Asian origin (Pakistani, Indian and Bangladeshi. F: free-living; I: institutionalised. Light grey bars are males and dark grey bars are females. Taken from Lanham-New, 2011 (42)

1.3 Common mental disorder

1.3.1 Definition of common mental disorder

The term “mental health” can be used in a number of ways. Mental well-being is a subjective concept involving perceptions of autonomy, and fulfilment of an individual’s intellectual and emotional potential. According to the WHO, mental health is not just the absence of a disorder. It is:

> *“A state of complete physical, mental and social well-being, and not merely the absence of disease” (57)*

Similarly, mental ill-health is not solely an absence of good mental health. Psychological distress is a common and normal reaction to some situations such as adverse life events. Reactions may become abnormal if they last a long time, are severe and have a damaging effect on individuals or others (1).

Research for the thesis was limited to CMDs, also known as adult neurotic disorders (1), encompassing conditions that:

> *“…cause appreciable emotional distress and interfere with daily function, but do not usually affect insight or cognition” (9)*

These disorders include depression, anxiety, phobias, and obsessive compulsive disorders. CMDs are usually diagnosed based on the presence of symptoms (**Box 1.1**). Symptoms of CMDs generally exist on a continuum whereby some individuals may be below the diagnostic threshold but are at very high risk of developing a disorder. Additionally, CMDs do not always present in discrete groups of symptoms for example, the presence of mixed anxiety depression is common.

Box 1.1 Example symptoms of CMDs (1)

- Low mood
- Fatigue
- Irritability
- Poor concentration
- Impaired sleep
- Impaired appetite
- Impaired libido
- Low self-esteem
- Feeling of worthlessness
- Suicidal ideas
- Palpitations
- Trembling
- Feeling of unreality
- Fear of dying
- Repetitive and compulsive thoughts and actions

1.3.2 Prevalence and treatment of common mental disorder

Mental and behavioural disorders are a leading cause of disability and disease burden worldwide (58). They affect people in all regions, in all societies and across all stages of the life-course. Globally, approximately 450 million individuals suffer from mental and behavioural disorders in their lifetime (59). The WHO found depression to be the leading cause of disability as measured by Years Lived with Disability (YLDs) and the second cause of Disability Adjusted Life Years (DALYs) for people aged 15-44 years (60).

In 2007, prevalence of CMDs in England was assessed using the *Clinical Interview Schedule-Revised* (CIS-R) (9). This study observed that 15.1% of adults aged 16 years or over had significant neurotic symptoms (CIS-R score ≥12), whereby 7.5% of these had severe symptoms (CIS-R >17) requiring treatment. Additional investigation of specific disorders found that 16.2% of adults met the diagnostic criteria for a least one CMD. This is higher than the

overall CIS-R as it is possible to meet the threshold for some types of CMD without having a total CIS-R score of 12. More than half of those with CMDs had mixed anxiety and depressive disorder (9%), 4.4% had generalised anxiety disorder, 2.3% depression and less than 1.5% met the diagnostic criteria for the remaining categories (9). Women and those aged 45-54 years were found to be most likely to have a CIS-R score of ≥12 (9).

In a US study, the median age of onset of an anxiety disorder (as measured by the *Diagnostic and Statistical Manual IV* (DSM-IV)) was 11 years and mood disorder 30 years, with later onsets usually consisting of co-morbid conditions (61). Although CMDs can affect individuals at any time point, it has been shown that those in mid-adulthood were more likely to present with significant symptoms of CMDs (9).

CMDs, particularly depression and anxiety, can have a lifelong course consisting of relapse and remission. For example, a national longitudinal survey found that 51% of women and 49% of men who had a CMD at baseline still had the disorder 18 months later (62). Factors that contributed to recovery included lower baseline symptom score, higher socioeconomic position, higher social support and less adverse life events (1).

The treatment of mental and behavioural disorders has evolved through time (59). Historically, individuals with mental illness were isolated from society and confined to asylums. As the medical model of mental illness developed, individuals were treated with newly identified drugs. Insights into the social components of mental health has contributed to the way in which disorders are treated today (59).

There is considerable variation in severity and type of CMDs, therefore treatment can be difficult. The most common treatment in primary care is psychotropic medication, however the National Institute of Health and Care Excellence (NICE) recommend the use of other psychological treatments such as cognitive behavioural therapy or counselling in combination with medication (63). Although the vast majority of cases of CMDs that are diagnosed by general practitioners (GPs) are treated in primary care, many individuals do not

seek treatment. This could be due to under-recognition for mild disorders and/or the avoidance of individual patients to report their symptoms (1, 63).

The WHO's comprehensive mental health action plan 2013-2020 adopted during the 66th World Health Assembly outlined a number of new directions for the treatment of mental illness (64). These plans include movement away from a medical model of mental illness to the provision of community based care while addressing social determinants of mental health (64).

In order to reduce the burden of CMDs in the population, the multidimensional and interactive risk factors need to be addressed.

1.3.3 Risk factors for common mental disorders

There are numerous factors that can interact to maintain mental health or influence mental ill health. These factors can be on the structural (e.g. access to healthcare), community (e.g. social networks) and individual level (e.g. psychological factors) (1). **Figure 1.3**, from the *Foresight report, Mental Health: Future Challenges*, illustrates the complexity of these mechanisms (1). Further details on the complex and inter-linking biological, psychological, social and lifestyle factors related to CMDs will be discussed in this section.

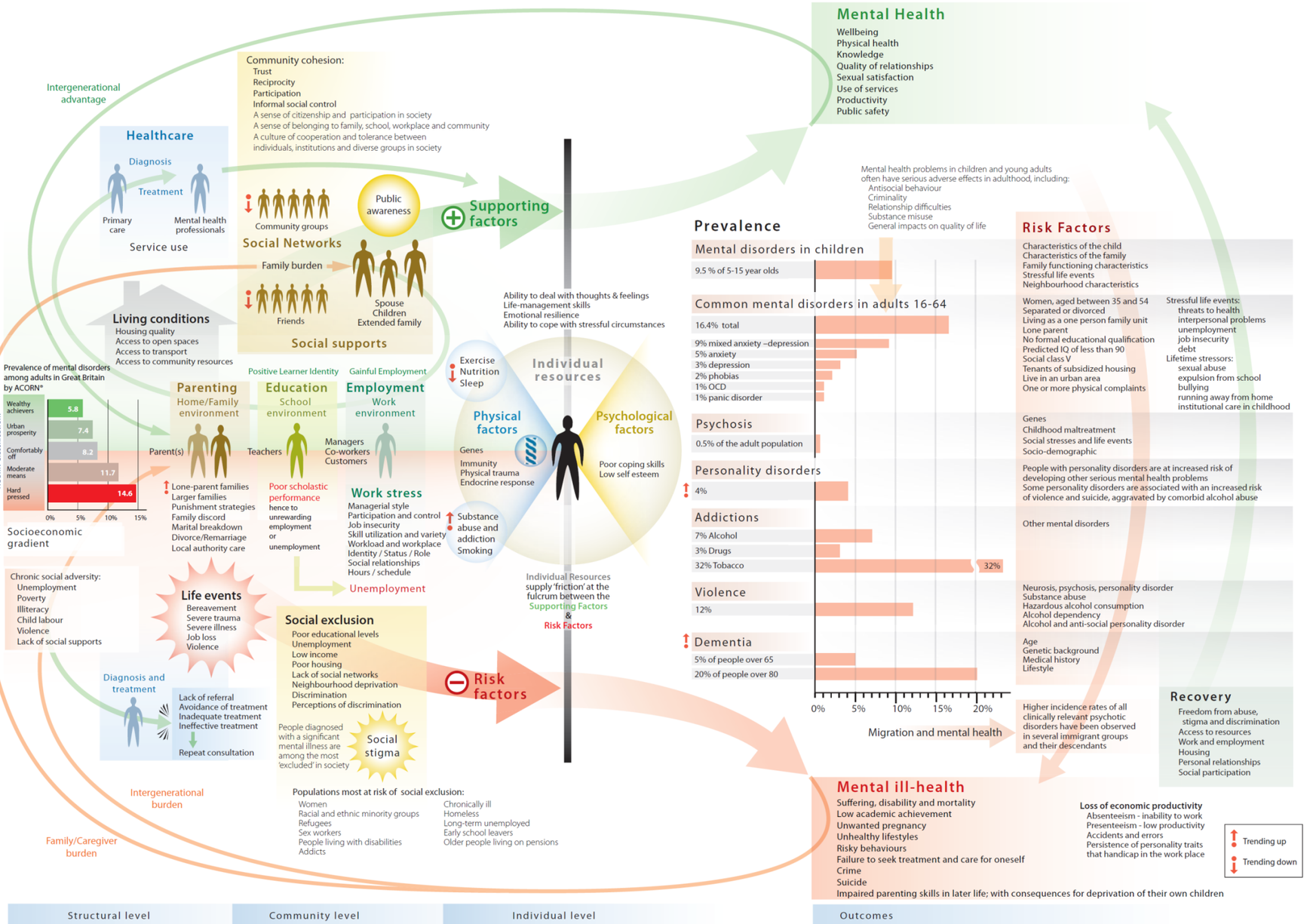

Figure 1.3: Factors associated with mental health

Taken from Foresight report, 2008 (1)

Biological factors

Significant advances in neuroscience have amplified our understanding of the brain. The brain is an extremely complex organ that integrates genetic, biochemical and molecular information with information from the outside world. The main cells of the brain include neurons, which send and receive information, and neuroglia which protect neurons. Each neuron communicates with others via specialised chemicals (neurotransmitters) through structures (synapses). Synaptic connections can be created and remodelled to change the structure of the brain throughout the life-course (65). Mental and behavioural processes are the result of the development of circuits between neurons (59). Biological mechanisms for CMDs are not fully understood, however, there are a number of proposed models. They include the disruption or deficiency of a group of neurotransmitters called catecholamines (dopamine, noradrenaline and serotonin) (66), disruption of hormonal function for example, the stress hormone cortisol (67) and changes in the function of the immune and central nervous system (66, 68).

Psychological factors

Childhood and adolescence are sensitive times for the development of emotional disorders (59). Many CMDs in adulthood begin in childhood. For example, internalising and externalising disorders at ages 7, 11 and 16 years have been associated with a higher risk for mid-life anxiety and affective disorders (69). Early life factors such as major psychological stressors and trauma can influence CMDs in later life (70). Furthermore, the relationship between a child and their parents or caregivers is an essential determinant of mental and behavioural development (71). Individuals are more likely to engage in behaviours where they are rewarded and learn how to cope with stressful life events through this caregiver interaction. Thus, a disorder can be viewed as maladaptive behaviour that has been learned (59). Cognitive functioning may also have a role in CMDs (***section 1.4.3***), with one study suggesting that higher childhood cognitive ability is associated with fewer symptoms of anxiety and depression in adulthood (72).

Social factors

Socioeconomic factors including poverty, unemployment and low education have also been associated with CMDs (61). Longitudinal studies of British participants have shown that socioeconomic position (SEP) in childhood and adulthood can influence depression and anxiety in mid-life (73, 74). This relationship is complex as it remains unknown if poverty is the cause of a disorder or if those with a disorder will drift into poverty (59).

Lifestyle factors

Health-related behaviours throughout life also influence the onset and course of a CMD. For example, the use of substances such as tobacco and alcohol has been associated with CMDs. Epidemiological studies have identified that both non-drinkers and harmful drinkers have higher levels of CMDs compared with moderate drinkers (75-78). Additionally, those with a mental disorder are approximately twice as likely to smoke compared to those without a disorder (79). Nicotine is an addictive substance that has an effect on the brain, therefore it remains unknown whether those with a CMD smoke more to alleviate their symptoms or if smoking causes a CMD (59).

Physical inactivity is another important factor in CMDs. A study of British individuals found a consistent association between physical activity in adolescence and psychological well-being in adulthood (80). Furthermore, literature reviews provide evidence that exercise appears to be beneficial in the treatment of CMDs in combination with other medication (81, 82). A link between CMDs and obesity has been suggested by a meta-analysis conducted in 2010 (83), with further studies suggesting that a U-shaped trend exists between BMI and depression (84). CMDs may cause obesity via changes in eating behaviour or reduced physical activity; conversely, obesity may cause CMDs for example through a negative body image.

Good nutrition is essential for the maintenance of both physical and mental health. A number of specific nutrients have been associated with mental health. For example, low maternal folate status during early pregnancy has been associated with a higher risk of emotional problems in childhood (85). Dietary

folate has also been associated with depressive symptoms in middle-aged men (86) and poor thiamine status has been associated with higher odds of depressive symptoms (87, 88). Despite some evidence, the causal association between specific nutrients and CMDs remain equivocal. A recent meta-analysis suggested that a diet consistent with high intakes of fruit, vegetables, fish and whole grains may be associated with a reduced depression risk (89).

There are a multitude of interlinking risk factors affecting CMDs, of which only a few have been discussed in this section. CMDs can occur in the absence of stressful and negative life events, however, they are more likely to present following an incident (66). Genetics (90), emotional reactivity, presence of chronic illness (91, 92), low self-esteem (93), poverty, work stress (94), poor interpersonal relationships, family history, gender, age, marital status, unemployment and social cohesiveness (91, 95, 96) are some of the other factors related to onset and progression of CMDs.

1.3.4 Public health importance

If people with CMDs are untreated they are more likely to experience long-term disability and premature mortality (97). CMDs can have a large impact on individuals, families and communities. Individuals suffer the symptoms of a CMD and are often unable to participate in work and leisure activities while families often bear the burden of care via both emotional and financial support.

The high prevalence of CMD means the cumulative cost to society is great, for example, causing one fifth of days to be lost from work (98). Therefore, research into novel approaches for prevention and/or treatment is essential.

1.4 Cognition

The second outcome to be investigated is cognitive function. The process of normal ageing is not well characterised and differences in cognitive function in mid-life may reflect some pre-clinical features of later-life pathological conditions (99). Therefore, cognitive disorders at older ages are mentioned briefly in this section.

1.4.1 Definition of cognition

Cognition refers to mental processing. Through these processes, the brain acquires, stores, transforms and applies information. Cognitive processes include various mechanisms such as attention, perception, language, organisation, problem solving, memory and decision-making amongst others (100) and are often organised into specific domains. Examples of cognitive domains include, executive function (i.e. the ability to plan, solve problems, shift focus and perform abstract reasoning), memory (i.e. ability to learn and recall information) or visuospatial ability (i.e. comprehension and effective manipulation of nonverbal information) amongst others.

Traditionally, cognitive function has been studied in the distinct fields of cognitive development and cognitive ageing, however cognitive changes can occur throughout the whole lifespan (101). Cognitive function is a dynamic process which can be influenced by many genetic, environmental, social and biological interactions throughout the lifespan (102).

In their 2006 paper, Craik and Bialystok proposed a framework consisting of representations, control and their interaction to describe cognition throughout the lifespan (101). Representations consist of crystallized schemas for example, acquired knowledge or experience and vocabulary. Control consists of fluid operations such as memory, name-finding, complex decision making and speed of processing. The general assumption is that representation (or crystallised pragmatics) increases during childhood and remains stable in later life whereas control (or fluid mechanics) increase during childhood, reach a threshold in early-adulthood and decline with increasing age (101, 103). These life-course changes are depicted in **Figure 1.4**.

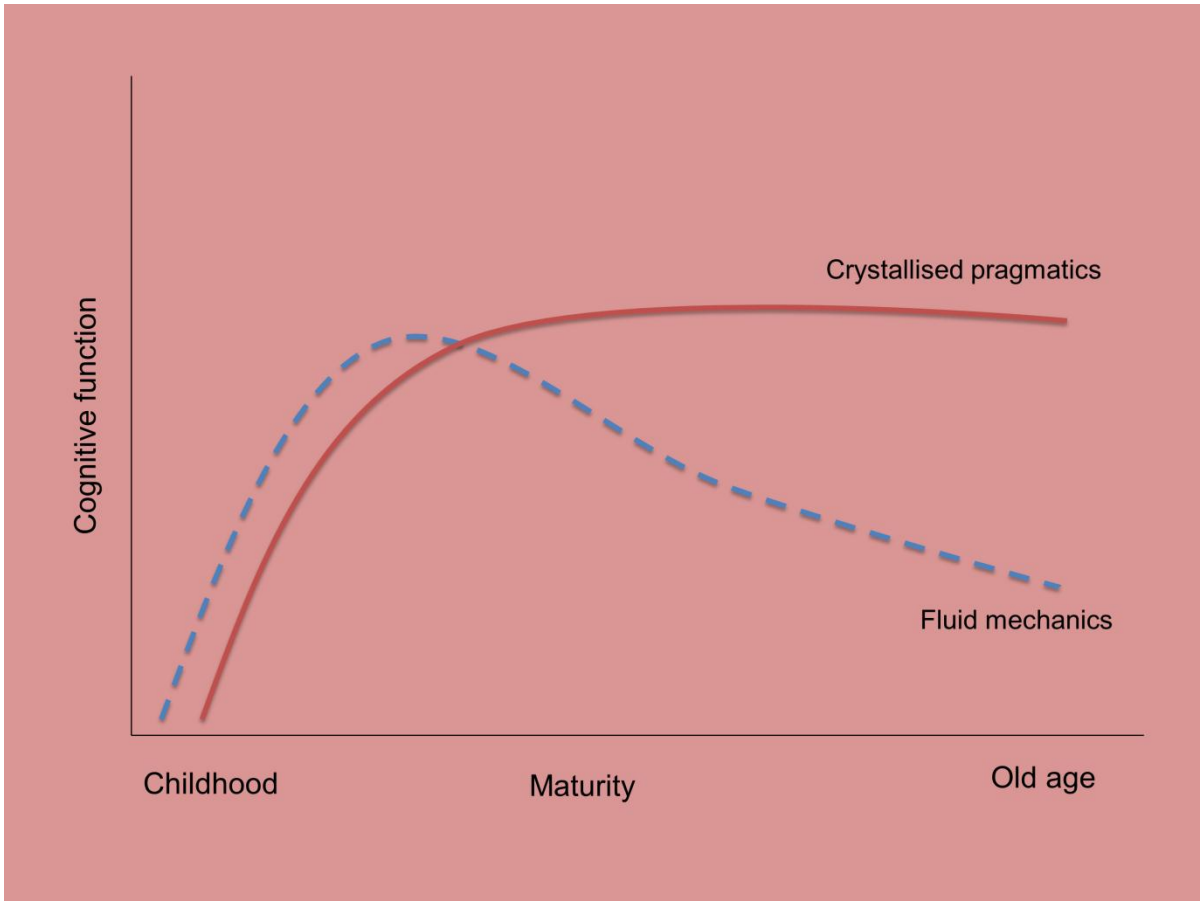

Figure 1.4: Cognitive function throughout the lifespan

Adapted from Craik, 2006 (101)

Determining the age at which normal age-related cognitive decline begins remains complex. It is difficult to separate the effects of normal ageing and pathological processes on cognitive function. Furthermore, there are differences in findings from longitudinal and cross-sectional studies. Longitudinal studies tend to find little evidence for age-related decline before the age of 60 years, whereas cross-sectional studies find linear declines across the lifespan starting in young adulthood (104, 105) (**Figure 1.5**). Cross-sectional studies may overestimate the age at which cognitive decline occurs due to cohort differences (i.e. differences in cultural factors, economic conditions etc.). On the other hand, longitudinal studies may underestimate the age at which cognitive decline occurs due to practice effects (i.e. the influence of past experience of taking a test) and attrition (105). Therefore, it is plausible that age-related cognitive decline can emerge in mid-life, before the age of 60 years.

1.4.2 Cognitive disorders in later life

Cognitive ability varies widely among middle aged and older people, with maintained function at one extreme and dementia at the other (106, 107). Cognitive impairment is evident when decline is greater than what is expected for age and education level (108). Although a clinical definition for cognitive impairment is still being debated, a score of 0.5 on the clinical dementia rating scale, or stage 3 on the global deterioration scale for ageing and dementia, has been used in the past. Individuals with the amnestic sub-type of impairment (i.e. problems with memory) have a higher risk of progression to Alzheimer's disease (AD) (108). There are a number of different types of dementia, including mixed dementia. It has been estimated that approximately 50-70% of all cases of dementia are the result of Alzheimer's disease and this number may increase with age (109). Other types of dementia include vascular dementia, frontotemporal dementia and dementia with Lewy bodies, affecting various aspects of cognitive function (110).

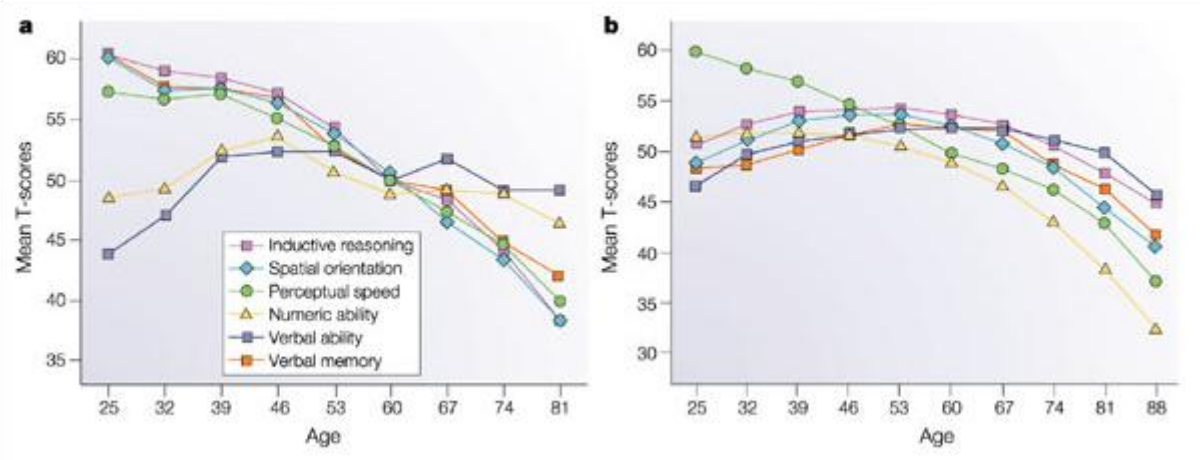

Figure 1.5: Cross-sectional and longitudinal estimates of age-relates change in cognitive function

A: Cross-sectional data from the Seattle Longitudinal Study. B: Seven year longitudinal data from the same study. Taken from Hedden, 2004 (105)

1.4.3 Factors influencing cognition function

As with CMDs, there are numerous interacting factors that influence cognitive function, some of which will be discussed in this section.

During normal senescence, natural reductions in brain tissue volume and other anatomical changes contribute to the decline in cognitive function (105). However there are several factors throughout the lifespan including genetic, environmental, socioeconomic and biological which may affect brain structure and function and influence an individual's cognitive ability at any time point.

Since the brain remains plastic throughout the life-course (65), it is no surprise that health, social and environmental factors in early life can have a dramatic effect on outcomes in later life (111). Cognitive ability in childhood has been shown to be a strong predictor of cognitive ability in adulthood (112). Childhood cognitive ability has also been associated with a myriad of health and social outcomes in later life including socioeconomic position (SEP), educational attainment, health behaviours, and emotional status (111) which in turn can influence cognitive function in mid- to later life. Further studies have demonstrated an association of lower childhood SEP with poorer cognitive function in adulthood (113, 114). Another study suggested an indirect effect of childhood SEP on cognitive function via its effect on later measures of SEP (115). Additionally, SEP in adulthood as well as educational attainment throughout the life-course has been found to influence cognitive function in adulthood (113, 116-119). Early adverse conditions have been shown to influence cognitive ability in childhood and adolescence and in some cases are associated with cognitive ability in mid-life (120).

Mental health status may be associated with cognitive function (121), with some evidence for an association of depression with dementia, particularly AD and vascular dementia (122). However, CMDs and cognitive impairment often co-occur making the causal direction difficult to determine. In a recent paper Richards *et al* propose three possible explanations for the association between affective disorders (for example, depression and anxiety) and cognitive impairment (123). Firstly, affective disorders may directly cause cognitive

impairment; secondly, affective disorders are the result of an emotional response to cognitive impairment; thirdly, affective disorders may share similar neuropathological pathways to cognitive impairment. Depression can often lead to increased levels of glucocorticoid (also released in the presence of chronic stress) production which in turn could cause hippocampal atrophy (associated with memory loss). On the other hand, cognitive impairment with damage to the frontostriatal brain or with hippocampal atrophy may result in depression (124). One study suggested that depressive symptoms were associated with memory decline amongst an older population (121). However, a study conducted in a relatively younger population (60-64 years) found no inverse association between symptoms of affective disorder and cognitive test scores (123), implying that associations may be more likely amongst older populations. Another study found that social integration protected against memory decline (125).

In their review, Hughes and Ganguli identified factors from low birth weight and fetal under nutrition to obesity and occupation as potential modifiable risk factors for cognitive impairment in later life (126). Smoking has been associated with faster declines in verbal memory and with slower processing speed (127). Furthermore, passive smoking has been associated with higher odds of cognitive impairment (128). The relationship between alcohol consumption and cognitive function is more complex. Compared with abstinence, alcohol consumption in older adults has been associated with better cognitive ability (129). Furthermore, alcohol consumption has been associated with a slower memory decline from 43 to 53 years in men but with a more rapid decline in processing speed in women (130). Other risk factors that may affect cognitive function in mid to later life include, vascular health, for example, high blood pressure (126, 131, 132), obesity (133) and physical fitness (134). Physical function and cognitive function are associated, however the direction of their relationship remains unknown (135). Physical exercise as well as leisure-time activity may be beneficial to memory in mid-life, however their mechanisms could involve several different pathways (136).

Nutrition has been associated with cognitive function. For example, iron deficiency anaemia had been negatively associated with cognitive performance

in childhood (137) and vitamin B-12 serum concentrations positively associated with cognitive function in adults (138). There is evidence that high fish consumption is associated with reduced cognitive decline in later life (139), however evidence from a randomised controlled trail with omega-3 long chain polyunsaturated fatty acids did not support this (140). An animal study has shown a diet rich in antioxidants may delay cognitive ageing (141). In addition to single nutrients, healthy dietary patterns have been implicated in slower declines in cognitive function. For example, adherence to the Mediterranean diet was associated with slower cognitive decline and a reduced risk of Alzheimer's disease (142) and diets with high intakes of fruit and vegetables, complex carbohydrates and cereal in early mid-life may be protective of verbal memory decline from mid- to later-life (143). However, it remains unknown if a nutritional deficit is the cause or consequence of reduced cognitive function (144).

Genetic factors may also play a significant role in determining an individual's cognitive ability. For example, mutations of apolipoprotein E alleles have been shown to be associated with cognitive performance (145) and are strong predictors of Alzheimer's disease (146-148). The association between apolipoprotein E and cognitive function will be discussed further in **Chapter 7**.

1.4.4 Public health importance

Global populations are predicted to increase in number and shift towards an older demographic (10). Therefore, there is a growing interest in understanding the factors that lead to healthy ageing. Poor cognitive function is linked with a higher risk of dementia, disability and consequently institutionalisation and mortality (149, 150). The WHO estimated that 35.6 million people globally have dementia, with this number projected to double every 20 years (151). The Alzheimer's society 2012 estimates suggest 800,000 people in the UK had a form of dementia (152). Furthermore, in a study conducted using data from the Cognitive Function and Ageing Study II in 2011, 670,000 adults aged ≥65 years were estimated to have dementia (153). According to the WHO, the social and economic implications of dementia cost 1.24% of gross domestic product in high-income countries (151). It is difficult to estimate the public health impact of

reduced cognitive function in mid-life, before the onset of cognitive disorders. According to Fries' compression of morbidity hypothesis, if the age of onset of cognitive decline can be postponed to a shorter period before the time of death (154), quality of life of individuals could be significantly improved. While most interventions to target a decline in cognitive ability are aimed at people aged 60 and over, there is some evidence to suggest that decline may occur prior to this, highlighting the need for early intervention.

1.5 Rationale

The importance of maintaining mental health and cognitive ability has been emphasised during this chapter. Vitamin D has been a candidate in the effort to understand how novel modifiable exposures may influence these outcomes.

Evidence is accumulating in support of a biologically plausible relationship between vitamin D and the brain. Not only has $1,25(OH)_2D$ been shown to pass the blood-brain barrier (155), but recent data has also demonstrated the presence of vitamin D metabolites (156) and enzymes for bioactivation (1α-hydroxylases, 25-hydroxylases) (157-159) and catabolism (24-hydroxylase) (160) in the central nervous system. This supports the potential existence of localised metabolic pathways for $1,25(OH)_2D$ in the brain. The additional finding of VDR in various areas of the human brain, including in key behaviour regulation sites (amygdala, cortex and cerebellum), has contributed to the suggestion that $1,25(OH)_2D$ has neurosteroid properties (158, 161-164).

$1,25(OH)_2D$ has many potential roles in the neuroendocrine system. This hormone has been shown to play a role in brain development (160, 162, 165-167), neuroprotection (168-172), have immunosuppressant effects (163, 173, 174) and anti-depressant or behavioural modification functions (175-177). $1,25(OH)_2D$ may influence cognitive function via its impact on vascular health i.e. through influencing blood pressure (178, 179), stroke (180) or diabetes (179, 181). $1,25(OH)_2D$ has also been shown to aid in the prevention and clearance of amyloid-β plaques seen in AD (182, 183) and can protect against glucocorticoid-induced apoptosis in the hippocampal cells which is often the result of depressive disorders (168).

In addition to experimental studies, epidemiologic studies have been carried out to assess the association between vitamin D and CMDs and between vitamin D and cognitive performance. Although results remain equivocal, two meta-analyses have demonstrated an inverse association between 25(OH)D and depression (184) and cognitive performance (185). Examination of previous epidemiologic studies will be included in **Chapters 5** and **6**.

In this thesis, investigation of the association between vitamin D status CMDs and cognitive function will employ methods of observational and genetic epidemiology. The use of these various study designs will provide additional insight into the relationship between vitamin D status, CMDs and cognitive function. The aims are outlined in the next chapter.

1.6 Summary

- The maintenance of mental health and cognitive function are important public health issues
- Many factors from childhood and adulthood influence mental health and cognitive function in mid-life
- Vitamin D status has been associated with a variety of potential health outcomes and is affected by several environmental, lifestyle and genetic factors. Low concentrations of 25-hydroxyvitamin D is widespread
- Experimental and epidemiological evidence suggests an association between vitamin D, mental health and cognitive function
- This thesis endeavours to address the question: how is vitamin D status associated with common mental disorder and cognitive function in mid-life

1.7 Plan of thesis

- *Chapter 2* will outline the overall aim and specify the study objectives
- *Chapters 3* and *4* will discuss the different methodological approaches and data used to address the aim
- *Chapters 5* and *6* will describe the work and results from the observational study of vitamin D, common mental disorders and cognitive function
- *Chapters 7 and 8* will explore the use of genetic information to understand the role of vitamin D, using cognitive function as an example
- *Chapter 9* will present a final discussion and overview of the research

Papers published in peer-reviewed journals directly related to work in this thesis are provided in **Appendix 1**, including:

Paper I

Maddock J, Berry DJ, Geoffroy MC, Power C, Hyppönen E. 25-Vitamin D and common mental disorders in mid-life: Cross-sectional and prospective findings. *Clinical Nutrition* 2013; 32(5): 758-764.

Paper II

Maddock J, Geoffroy MC, Power C, Hyppönen E. 25-Hydroxyvitamin D and cognitive performance in mid-life. *British Journal of Nutrition* 2014; 111(5):904-914.

Paper III

Maddock J, Cavadino C, Power C, Hyppönen E. 25-Hydroxyvitamin D, *APOE* ε4 genotype and cognitive function: Findings from the 1958 British birth cohort. Submitted 2014.

Paper IV

Alfred T, Ben-Shlomo Y, Cooper R, Hardy R, Deary IJ, Elliott J, Harris SE, Hyppönen E, Kivimaki M, Kumari M, **Maddock J**, Power C, Starr JM, Kuh D, Day IN; HALCyon Study Team. Genetic variants influencing biomarkers of nutrition are not associated with cognitive capability in middle-aged and older adults. *Journal of Nutrition* 2013; 143(5):606-612.

First author oral and poster presentations are:

- **Maddock J**, A Cavadino, Power C, Hyppönen E et al. Investigating the effect of vitamin D status on cognitive function using a Mendelian randomisation approach. Oral presentation at Vitamin D and Human Health, April 2014. Recipient of Young Investigator Award
- **Maddock J**, Geoffroy MC, Power C, Hyppönen E. 25-Hydroxyvitamin D and cognitive performance in mid-life. Poster presentation at UCL Graduate school open day, March 2014
- **Maddock J**, Geoffroy MC, Power C, Hyppönen E. 25-Hydroxyvitamin D and cognitive performance in mid-life. Poster presentation at UCL Institute of Child Health open day, November 2013
- **Maddock J**, Berry DJ, Geoffroy MC, Power C, Hyppönen E. 25-Hydroxyvitamin D and common mental disorders in mid-life. Poster presentation at International Congress of Nutrition, September 2013
- **Maddock J**, Geoffroy MC, Power C, Hyppönen E. 25-Hydroxyvitamin D and cognitive performance in mid-life. Poster presentation at International Congress of Nutrition, September 2013
- **Maddock J**, Berry DJ, Geoffroy MC, Power C, Hyppönen E. Symptoms of common mental disorders and vitamin D: Findings from the 1958 British birth cohort. Poster presentation at UCL Graduate school open day, March 2012
- **Maddock J**, Berry DJ, Geoffroy MC, Power C, Hyppönen E. Symptoms of common mental disorders and vitamin D: Findings from the 1958 British birth cohort. Poster presentation at UCL Institute of Child Health open day, November 2011. Recipient of 2nd place prize

Chapter 2 Aims and objectives

The overall aim of this thesis is to determine whether and the extent to which 25-hydroxyvitamin D concentrations affect common mental disorders and cognitive function. Using methods from both observational and genetic epidemiology and data collected mainly from a large nationwide cohort study, the 1958 British birth cohort, the aim will be achieved by:

I. Examining the cross-sectional and prospective association between 25-hydroxyvitamin D and prevalence of common mental disorders and determining the extent to which it may be explained by vitamin D related lifestyle factors
II. Examining the prospective association between 25(OH)D and cognitive function in mid-life
III. Evaluating the interaction between 25(OH)D and *APOE* ε4 genotype in relation to the association between 25(OH)D and cognitive function using a gene-environment interaction analysis
IV. Evaluating the relationship between 25(OH)D and cognition using the genetic indicators for lifelong 25(OH)D status in a Mendelian randomisation analysis

Chapter 3 Methodological approaches

3.1 Introduction

Since the 19th century, the discipline of epidemiology has been used to study why and how often health problems occur in different groups of people (186). Epidemiological methods can be credited with the successful identification of the source of a devastating cholera break in London in 1854, providing evidence for the association between smoking and lung cancer in 1950 and identifying the fetal origins of adult disease, amongst many others (186). Investigations into the aetiology of diseases and disorders have been greatly advanced by the advent of the human genome project coupled with advances in molecular biology and genetic technology. Epidemiology has responded to these advances by integrating genetic knowledge into the discipline (187).

Both observational and genetic epidemiology were used to address the aims as outlined in **Chapter 2**. The use of both approaches endeavours to triangulate evidence to facilitate greater understanding and increase confidence in the findings. Details on the strengths and limitations of observational and genetic epidemiology and how they were used to address the aims of the thesis are described in this chapter.

3.2 Observational epidemiology

Experimental studies, particularly randomised, double-blind controlled trials (RCT), are considered the gold standard of research. They involve randomisation of a population into groups, for example, one which receives treatment (or exposure) and another which does not. In theory, by using this approach, any variation in the outcome of interest can be attributed to the treatment (188). However, due to ethical and financial limitations these are not always feasible. Therefore, other methods are required to examine associations between risk factors and disease.

Observational epidemiology involves non-experimental studies, for example, cohort, case-control and cross-sectional studies. In these, the investigator observes an exposure and outcome in a population without exerting any

influence (188). Observational epidemiology has led to many successful public health interventions for example, folic acid fortification in flour to reduce neural tube defect in the US (189). Conversely, there have been many high profile failures, for example, while observational results suggested that vitamin E was protective for cardiovascular disease and beta-carotene for cancer, evidence from RCTs does not support these claims (190).

A good quality observational study needs to be well-designed, powerful and reliable. Random sampling from the population is the ideal and sufficient numbers of participants need to be included to ensure adequate power (i.e. the probability that the study will be able to detect a true effect (191)). The reliability of a study is determined by its ability to reproduce the same results if it is repeated a number of times (192).

Valid causal inferences from observational studies can be difficult to make. Spurious associations and inaccurate estimates may result from chance random error, systematic bias or confounding. While random error cannot be completely eliminated, it can be minimised through well-designed studies. Certain systematic bias such as measurement error (i.e. the difference in measures of outcomes or exposures and their true value (193)) can be minimised through using valid, quality instruments to assess relevant variables. Furthermore, selection bias (i.e. when there is a difference between those included and those not included), can affect the representativeness of the study sample.

One of the most likely explanations for past failures of observational epidemiology is confounding (3). Confounders are factors that correlate with both the exposure and outcome and may explain all or part of the measure of an association (194), for example, season is associated with both vitamin D status and depression. Some confounding can be minimised by using statistical methods however, it is difficult to account for all possible factors that may confound the relationship between the exposure and outcome.

Well-designed cohort studies can facilitate the optimal use of observational epidemiological methods. These studies often contribute rich and detailed data

on exposures, outcome and potential confounders that have been collected in a large number of participants throughout their lifespan. Therefore, an in-depth, longitudinal examination between the exposure and outcome can be conducted.

This thesis uses cross-sectional and prospective study designs. A cross-sectional study involves the examination of an exposure and outcome at one time point. While these studies are useful to investigate the presence of an association between an exposure and outcome, the timing of events and inference of causality remains difficult (188). Prospective studies are longitudinal in design. These studies record exposures and identify an outcome in the future, providing a temporal dimension to the association (195).

Observational epidemiology was used to address aims I and II on the cross-sectional and prospective associations of 25-hydroxyvitamin D (25(OH)D) with outcomes of interest (**Chapter 2**).

3.3 Genetic epidemiology

Genetic epidemiology was used to complement findings from observational epidemiology. The two genetic epidemiological methods of gene-environment interaction and Mendelian randomisation (MR) are described in this section.

3.3.1 Gene-environment interaction

There is a general consensus that the vast majority of disease is due to complex relationships between genetic and environmental factors. While many studies have focused on investigating either genetic or environmental determinants, the interaction between genotypes and environmental exposures on complex health outcomes is becoming more evident (196).

David J Hunter described the rationale for the study of gene-environment interactions (GxE) in 2005 (197). Firstly, GxE may achieve a better estimate of the population-attributable risk for both genetic and environmental risk factors by accounting for their joint interactions. Secondly, GxE may strengthen the associations between environmental factors and diseases by examining genetically susceptible individuals. Thirdly, GxE may aid in dissecting disease

mechanisms and finally, results from GxE may offer tailored preventative advice.

Figure 3.1 demonstrates the growing interest in this field through illustrating the PubMed search results since 1990. GxE studies have produced new and important findings, for example, it has been suggested that the risk of cognitive decline is particularly high in *APOE* ε4 carriers who have untreated hypertension (198, 199). However, GxE findings do not always replicate, as demonstrated in a meta-analyses in 2009 examining the interaction between the serotonin transporter gene with stressful life events on the risk of depression (200). The study of GxE requires information on both elements of the relationship. While description of the genetic side may be relatively accurate, depending on genotyping techniques (197), a precise depiction of the environment aspect may be more difficult, due to, for example, measurement issues or other interactive environmental factors (201). Another issue common in many GxE studies is statistical power. It is thought that the sample size required to detect an interaction is at least four times the sample size that is needed to evaluate the main effect of each of the variables (197, 202).

Chapter 7 displays a GxE analyses which investigated a differential effect of an environmental exposure on a health outcome depending on the presence of genetic variants in unrelated individuals (197, 203). **Figure 3.2** illustrates the concept of this interaction or conditional independence (203).

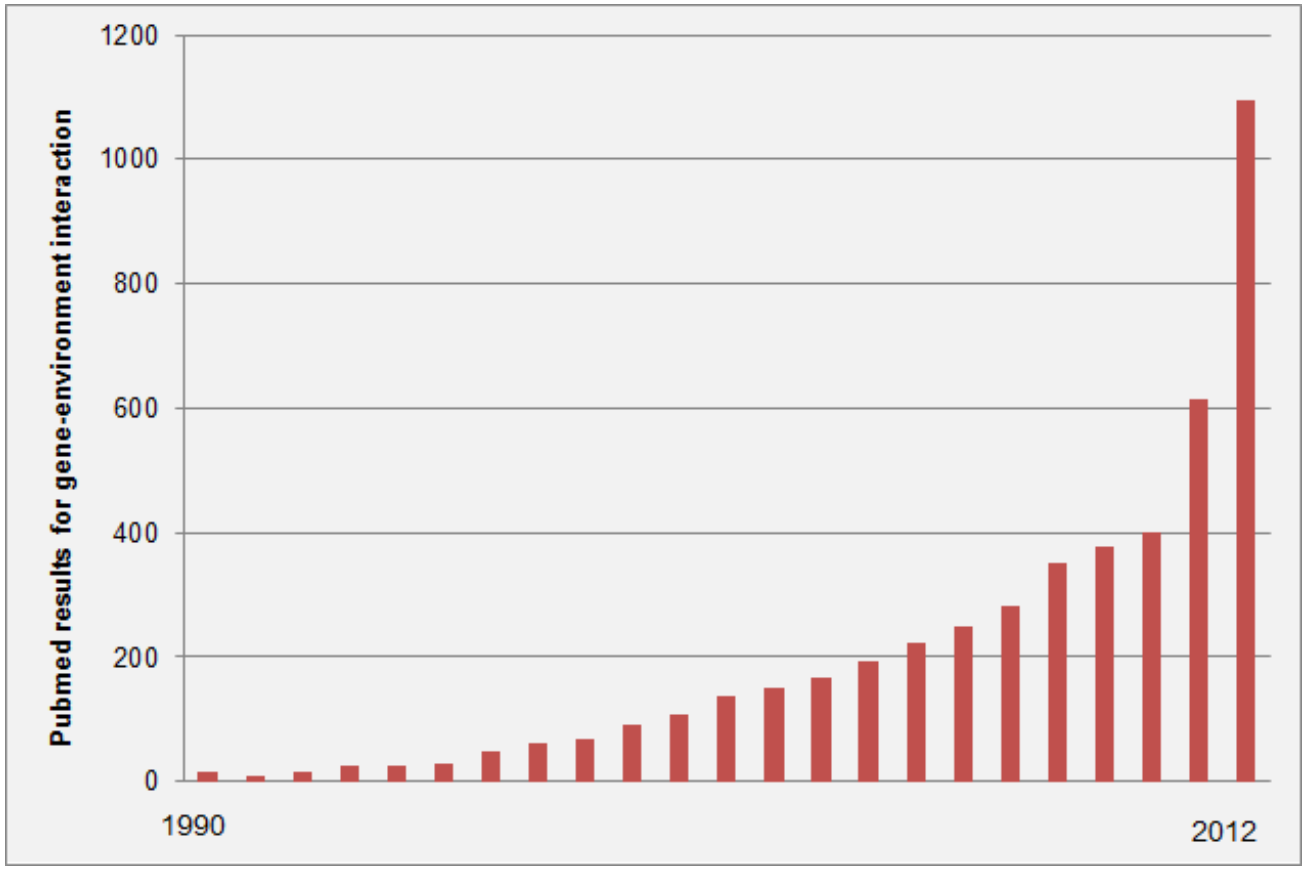

Figure 3.1: PubMed search results for *gene-environment interaction* between 1990 and 2012

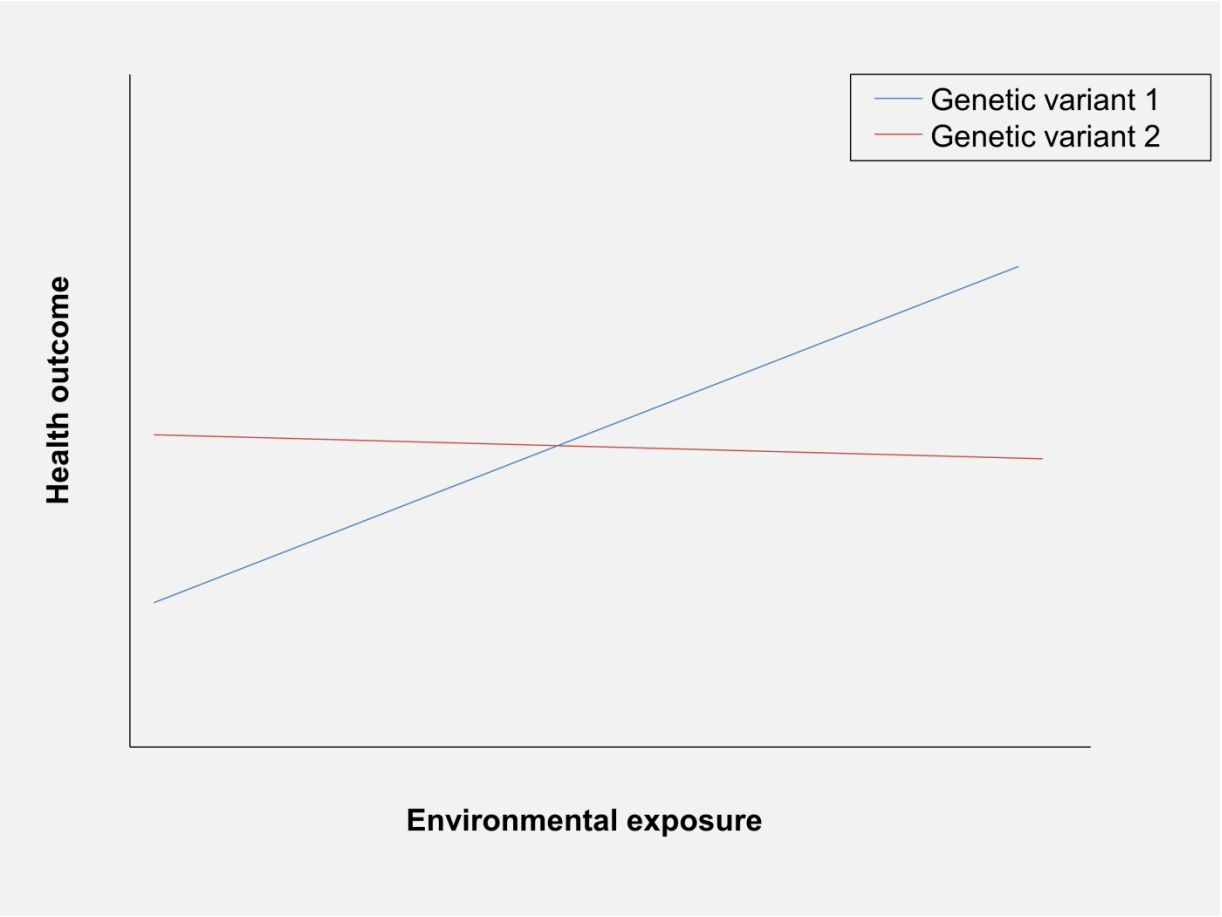

Figure 3.2: Theoretical gene-environment interaction on a health-related outcome

3.3.2 Mendelian randomisation

MR analysis is presented during **Chapter 8** to support observational findings. A glossary of genetic terms used can be found in **Appendix 2.1**.

MR is a method of genetic epidemiology that has been developed with the aim of overcoming some of the limitations of observational epidemiology (187). MR is based on an application of the method of instrumental variables (IV) commonly used in econometrics (3). The instrument is a variable that affects an outcome only through its ability to proxy an exposure. The aim of IV analysis is to estimate the causal effect of an exposure on an outcome. MR uses genetic variants as an IV.

MR can (in theory) overcome the issues of confounding and reverse causality that can be problematic for observational approaches. Genetic variants are transmitted from parents to offspring at random during meiosis. Therefore, factors that may confound the association between a modifiable exposure and outcome should be distributed evenly amongst those with and without the genetic variant, thus MR has been referred to as "nature's randomised trial", (**Figure 3.3**) (204, 205). Furthermore, since the genetic variant of interest is assigned prior to the appearance of outcome, the possibility of reverse causality is minimised (3).

In order to be effective, genetic variants must influence the exposure (in this case 25(OH)D status). If 25(OH)D concentrations are causally associated with cognitive function, genetic variants that proxy 25(OH)D concentrations should also be associated with cognitive function (205-207).

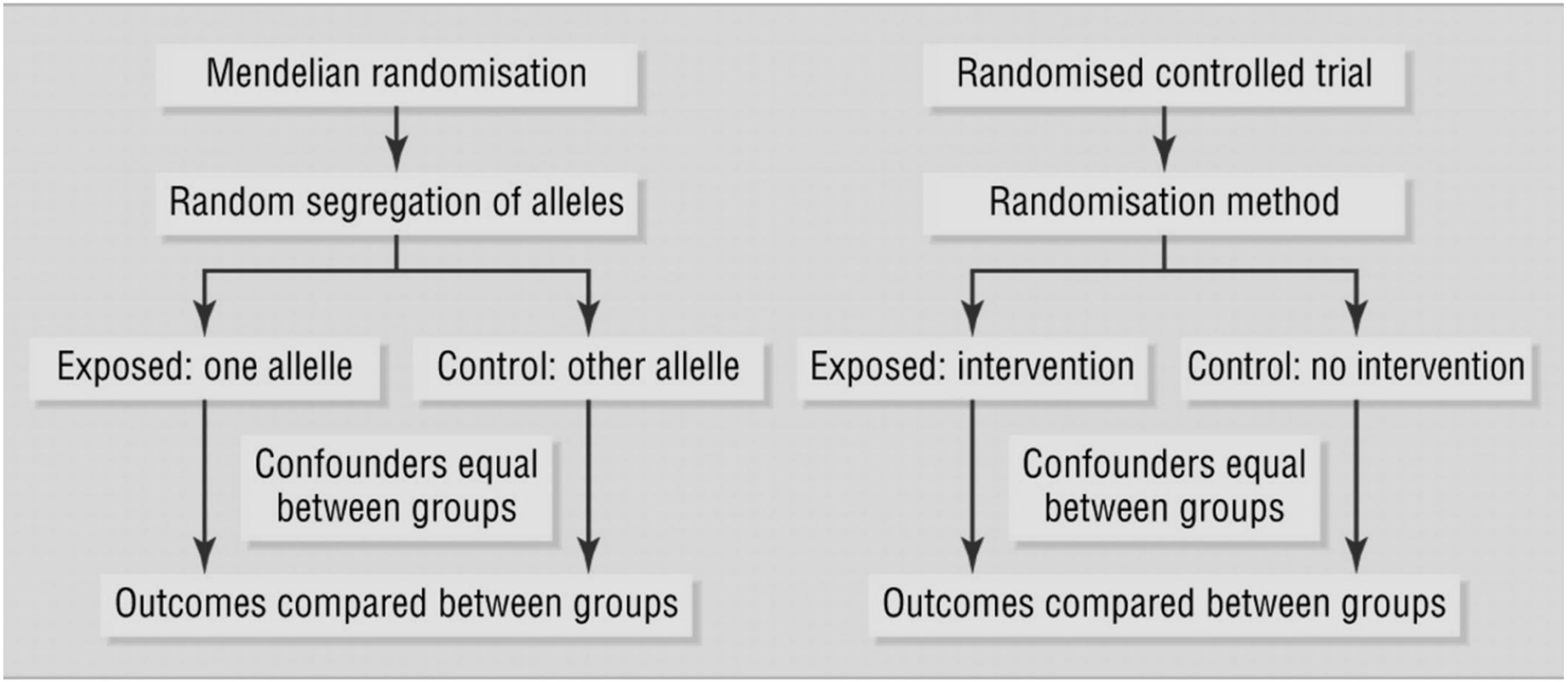

Figure 3.3: Comparison of design of MR studies with RCT

Taken from Davey-Smith, 2005 (205)

MR is subject to a number of limitations (3) and in order to be effective, the genetic variant must meet certain assumptions (**Figure 3.4**):

I. The genetic variant must be associated with the exposure
II. The genetic variant must be independent of confounding factors typically associated with findings from observational data
III. The genetic variant must not independently influence the outcome

While the association between the genetic variant and 25(OH)D concentration can be tested statistically, it is more difficult to test the other assumptions (206).

There are a number of factors that may result in spurious results from MR analysis (3, 206):

I. *Population stratification*

The genotype is not randomly allocated in the population. There may be systematic differences in allele frequencies between subpopulations, for example, due to non-random mating between groups. This can result in confounded associations that may be specific to only one population.

II. *Pleiotropy*

The genetic variant results in multiple biological outcomes. This is an issue if the resulting biological alterations affect the outcome through an independent mechanism.

III. Canalisation (or developmental compensation)

Developmental compensation may occur when environmental or other genetic factors offset the effects of the genetic variant. This will reduce the association noted between the genotype and exposure.

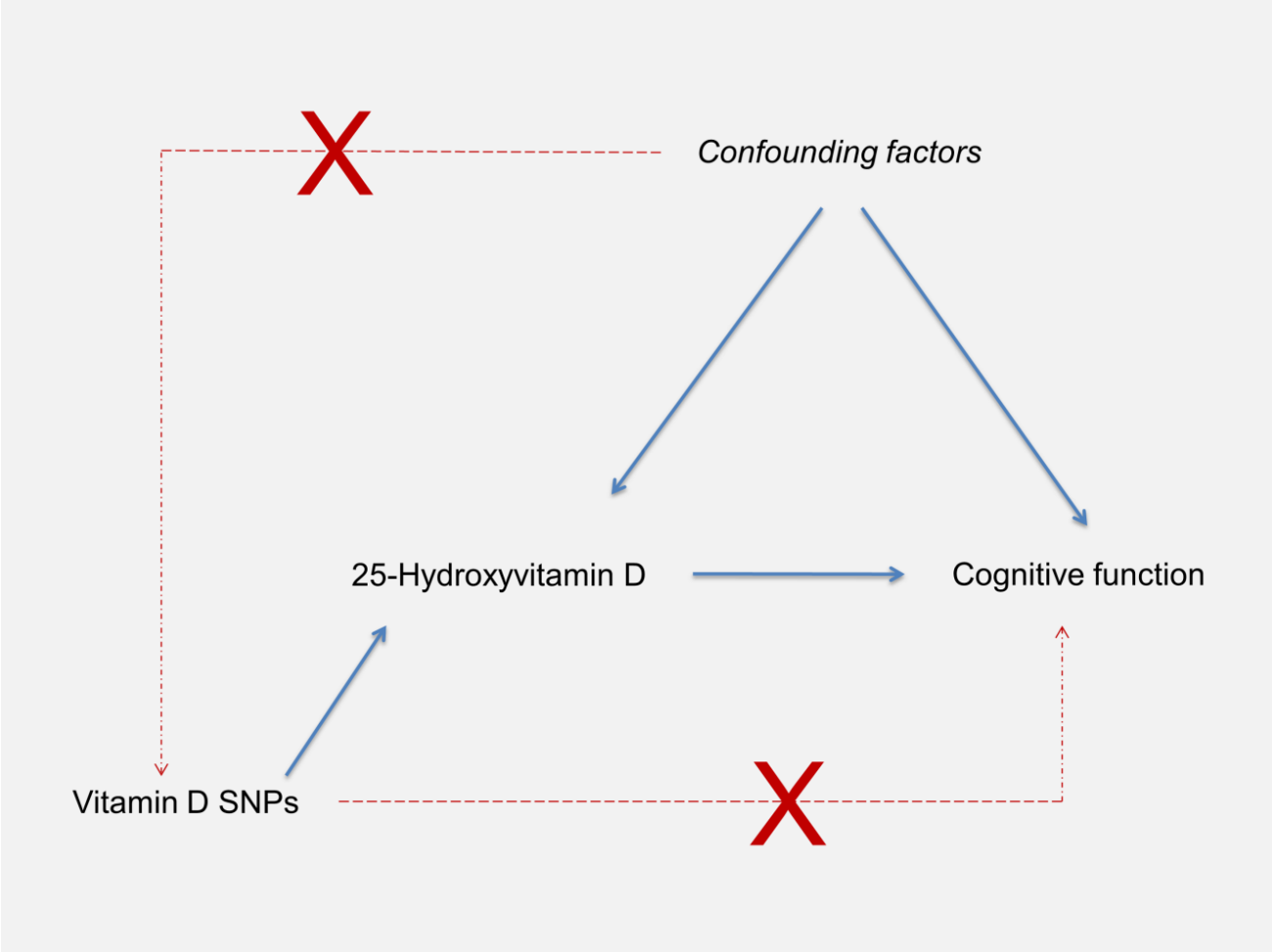

Figure 3.4: Assumptions of MR; illustrated by 25(OH)D and cognitive function

MR studies require large sample sizes partly due to the very small amount of variation in the exposure explained by genetic instruments (3). For example, a previous study found only 0.56% of the variation of 25(OH)D concentrations was explained by a combined genetic sore (207). Furthermore, an illustrative power calculation suggested that approximately 80,000 participants are required to establish a causal effect of vitamin D on blood pressure using a combined genetic sore (207). It is assumed that similarly large sample sizes are essential for the examination of the causal association between 25(OH)D and cognitive function. Therefore, a collaborative approach from other appropriate cohorts (**Chapter 4**) was taken.

Genetic epidemiology was used to address aims III and IV to further investigate the relationship between 25(OH)D and cognitive function (**Chapter 2**).

3.4 Statistical methods

This section gives an overview of the statistical methods applied in subsequent chapters. All statistical analyses were conducted using STATA version 12 (208).

3.4.1 Statistical methods for observational study

Systematic review

Two systematic reviews were conducted to identify, assess and compile primary evidence for the association of 25(OH)D with CMDs and cognitive function (**Chapters 5** and **6**). PubMed, which is an online version of Index Medicus produced by the US National Library of Medicine, was used to identify studies of interest. Search results were supplemented with relevant studies from review articles not captured by the PubMed search. Although methods of a systematic review were applied (209), there were some limitations. Quality of study appraisal may have been comprised since only one person conducted the review. Furthermore, results were restricted to studies in the English language, for which full test was available via online or University College London library and a formal assessment of publication bias was not conduced.

Descriptive statistics

Distributions of all main variables were examined using histograms and normality was assessed visually and using quantile-quantile (Q-Q) plots. A Q-Q plot examines how close the distribution of a variable is to a normal distribution. If continuous variables were found to be skewed, a natural logarithmic (ln) transformation was applied to improve approximation of a normal distribution. For example, 25(OH)D concentrations in all chapters were found to be slightly left skewed, therefore a natural logarithmic transformation was applied when 25(OH)D was used as an outcome. Where appropriate, tabulations of important covariates are displayed in relevant chapters. Pearson's chi-square tests were used to determine significant differences between categorical variables (210). Logistic and linear regression was applied to assess the association between covariates and outcomes as relevant.

Logistic regression models

Logistic regression was used to investigate the association between an exposure and a binary outcome in observational studies, for example, examining the association between 25(OH)D concentrations and presence of CMDs in **Chapter 5**. The estimate is expressed in terms of an odds ratio (OR). This describes the odds of having the outcome for every unit increase in the exposure. The 95% confidence interval (CI) is estimated and a Wald test is used to determine whether there is a significant association between the exposure and outcome (211). Potential confounding factors can be included in these models to examine whether there is an independent association between the exposure and outcome.

Linear regression models

Linear regression was used to investigate the association between an exposure and a continuous outcome, for example, examining the association between 25(OH)D and cognitive function in **Chapter 6**. The best-fitting straight line between the exposure and outcome is estimated using the method of least squares i.e. the values that minimise the sum of the squared vertical distance from the line. The estimate is expressed in terms of a regression coefficient.

95% CI are estimated and t-tests are applied to determine whether there is a significant association. In order to meet the underlying assumptions of linear regression, the outcome should be normally distributed. The distance between the observed values and corresponding point on the fitted line (i.e. residuals) should also be normally distributed. When the exposure is continuous, the regression coefficient is the increase/decrease in the outcome due to a unit increase in the exposure.

The effects of categorical exposures with more than two levels are estimated by creating proxy, *indicator variables* (212). These indicator variables are used to sort data into mutually exclusive categories whereby each category takes a particular value. A baseline group (usually coded as 0) is chosen to which the other groups (coded as 1, 2, 3 etc.) are to be compared. Each regression coefficient for non-baseline groups equal the differences in the mean outcome, compared to the baseline group. Evaluating the presence of a dose-response relationship, whereby the effect of the exposure increases (or decreases) in a systematic manner, is tested using a trend test. Here, the regression coefficient, known as the linear effect, is assumed to be constant and reflects the common change in log odds from one categorical level to the next across the entire range of the exposure (191). As for logistic regression, potential confounding factors can be included to examine whether there is an independent association between the exposure and outcome.

Non-linear regression models

As implied by the name, linear regression models allow a straight line to be fitted. In some cases, the relationship between the exposure and outcome may not be linear; therefore a polynomial curve can be examined. Non-linearity or curvature can be assessed by including a squared term of the exposure in the regression model (213). Non-linear regression models were assessed in observational studies.

Predicted values

Predicted values can be estimated after running either linear or logistic regression models. Predicted values give an estimate of the outcome for a

particular value of the exposure, based on the regression model. Predicted values were estimated and used to construct graphs in **Chapters 5** and **6**.

Interaction

When the effects of an exposure on an outcome vary by another factor, there is an interaction. Interaction can be assessed by including the multiplicative term of the two parameters (214). For example, **Chapter 6** examined whether the association between 25(OH)D and cognitive function varied for men and women. Where there was evidence for interaction, results were stratified. More complex interaction models were assessed in genetic studies, which will be described in the next section.

Missing Data

Missing data can be the result of attrition or item non-response. Attrition is common in longitudinal studies and involves a reduction in the numbers of participants in the study. Attrition could be due to participant mortality, refusal or inability to establish contact with the individual (215). Attrition may lead to bias and a reduction in the generalisability of the results if the reason for non-participation is not random. For example, in the 1958 British birth cohort (1958BC, which was used throughout this thesis) attrition to age 45 years was mostly through unavoidable loss, however, those with poor reading or maths score, with externalising or internalising behaviours and shorter stature were moderately under-represented (216). Therefore, inverse probability weighting was used in observational studies **(Chapters 5 and 6)** to correct for potential bias due to attrition.

Sample weights for **Chapters 5** and **6** were derived from previously identified variables that are underrepresented in the 1958BC (i.e. maths score at 7 and 11 years, externalising and internalising disorder at 7 and 11 years, gender, socioeconomic position (SEP), marital status and ability to pay household bills in the 1958BC). Weights were then be calculated in relation to the surviving cohort at 50 years (n=17,091) and used to adjust for selection bias during specified regression analyses.

Missing data can also be the result of a participant's non-response to particular items in the study. There are several different reasons for missing data which are outlined in **Box 3.1.** Missing data due to item non-response can lead to a reduction in the precision of estimates that result from the inevitably smaller sample size of complete case analysis (2).

Box 3.1 Missing Data mechanisms (2)

- ***Missing completely at random*** **(MCAR)**: There is no systematic difference between the missing value and observed values
- ***Missing at random (*****MAR):** The difference between the missing value and the observed value may be explained by differences in the observed measurements. Therefore, missing data is unrelated to the unobserved value itself after controlling for other variables in the analyses
- ***Missing not at random*** **(MNAR):** The missing values are dependent on unseen observations. Therefore, even after the observed data are taken into account, systematic differences between missing and observed values remain

Multiple imputation was used in the observational studies **(Chapters 5 and 6)** to account for missing covariates. For multiple imputation to be valid, the missing covariates should be MAR or MCAR. During imputation multiple copies of the dataset (n=10 in this case) are generated and missing values are replaced by imputed values using the chained equation method (MICE). As a single imputation does not account for the uncertainty in calculating missing values, imputed values are sampled from the predicted distribution based on the observed data. Following this, the model of interest, for example, linear regression models, is fitted to each imputed dataset. Results are combined and standard errors are calculated using Rubin's rules (217) which account for the variability in results between imputed datasets.

The associations of 25(OH)D with CMDs and 25(OH)D with cognitive function in observational studies, were assessed using complete, imputed and weighted datasets. The estimates obtained in each method were found to be broadly similar (see results in **Chapter 5** and **6**). Therefore, imputed regression results

are presented with completed and weighted results displayed in **Appendices 3** and **4**.

Additional and sensitivity analyses

In addition to the main analyses, **Chapter 5** explored the possibility of reverse causality by using linear regression models where naturally log-transformed 25(OH)D is used as the outcome and CMDs as the exposure. **Chapter 5** also assessed the association between 25(OH)D and different levels of severity of depressive symptoms by using different cut-offs for depressive symptoms in prospective analysis.

In **Chapter 6**, the association between 25(OH)D and cognitive function was additionally adjusted for the use of any nutritional supplement. This sensitivity analysis examines if other nutrients may play a role the in the association between 25(OH)D and cognitive function.

3.4.2 Statistical methods for genetic study

Linear, logistic and non-linear regression models described in ***section 3.4.1*** were also applied to genetic studies (***section 3.4.1***). This section gives an overview of the statistics used in GxE and MR analyses.

Gene-environment interaction

Effect modification (or interaction, ***section 3.4.1***.) is an important factor to consider when investigating the association between an exposure and outcome and is the main focus of **Chapter 7.** In **Chapter 7**, effect modification by *APOE* ε4 was examined from the most complex models, which tests for interaction between *APOE* ε4 and the quadratic term of 25(OH)D (i.e. $25(OH)D^2$). In order to determine the most appropriate model, *APOE* ε4 interaction was assessed in three ways:

1) Three way interaction between 25(OH)D, *APOE* ε4 and $25(OH)D^2$
2) Two way interaction between 25(OH)D and *APOE* ε4, adjusting for $25(OH)D^2$

3) Two way interaction between 25(OH)D and *APOE* ε4

The most appropriate interaction model was chosen by omitting the highest level term which did not make a significant contribution to the model (judged by *p*-value >0.05).

Mendelian randomisation

There are three main steps for the MR analyses used in **Chapter 8**:

I. Genetic variant association with exposure (i.e. 25(OH)D)
II. Genetic variant association with outcome (i.e. cognitive function)
III. IV ratio calculation

Linear regression models are used to assess the change in naturally log-transformed 25(OH)D according to the average increase in the number of effect alleles in the genetic variants. Linear regression models are also applied when the association between genetic variants with global and memory cognitive function is examined.

In order to estimate the causal effect of 25(OH)D on cognitive function, the IV ratio method is used (3). This MR estimate can be conceptualised as a ratio of two estimates i.e. the coefficient for the association between the genetic variant and 25(OH)D, and the coefficient from the linear regression of the genetic variant on cognitive function (218). The Wald or ratio estimator of the average causal effect for a one unit difference in X (i.e. 25(OH)D) on Y (i.e. cognitive function) is defined as $\hat{\beta}_{zy}/\hat{\beta}_{zx}$ where $\hat{\beta}_{zy}$ and $\hat{\beta}_{zx}$ are the coefficients from linear regressions of Y on Z and X on Z (i.e. genetic variant) respectively (**Figure 3.5**). The IV ratio is defined as: $\beta_{IV} = \frac{\beta_{zy}}{\beta_{zx}}$.

The variance of the IV ratio is estimated using the delta method, assuming that the covariance is close to zero (219):

$$Var(\beta_{IV}) = \frac{\sigma_{zy}^2}{\beta_{zx}^2} + \sigma_{zy}^2\left(\frac{\beta_{zy}^2}{\beta_{zx}^4}\right)$$

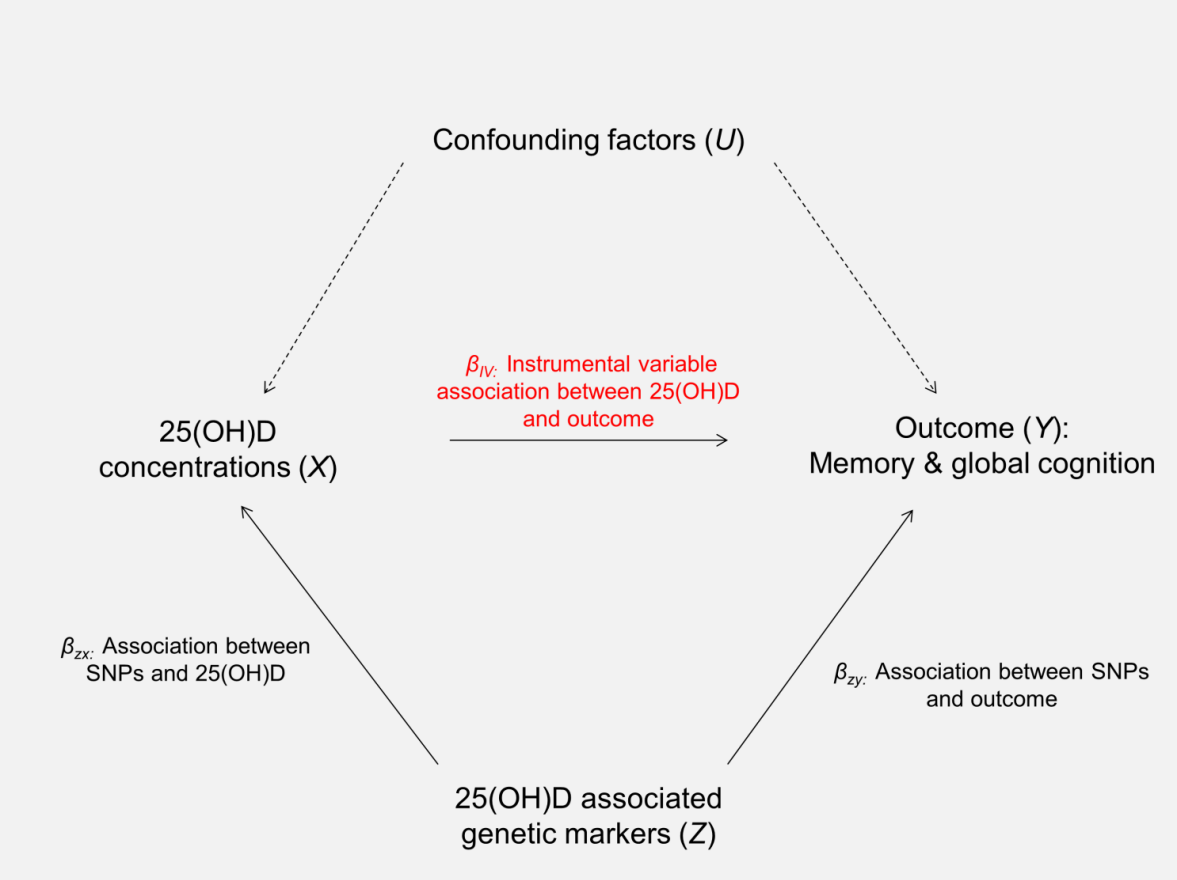

Figure 3.5: IV ratio estimation; illustrated by 25(OH)D and cognitive function

The IV ratio should be directly comparable with the more formal 2-stage least squares method (220). The advantage of using the IV ratio was demonstrated by Pierce and Burgess using simulated data sets (218). They found that there was no loss of statistical power or bias for the MR estimate when exposure data were available for only subset of participants, providing a good quality IV was used.

MR instrument validation

There are a variety of assumptions that underlie MR studies (outlined in ***section 3.3.2***). These state that the selected genetic variant should be linearly associated with the exposure of interest, be independent of confounding factors and must not be associated directly with the outcome of interest.

The strength of the genetic variant as an instrument for MR studies can be estimated from the adjusted R^2 which describes the proportion of variation in the exposure (i.e. 25(OH)D) explained by the genetic variant, adjusted for the number of parameters in the linear regression model. The F-statistic for the genetic variant can also be used to indicate the strength of the genetic variant as an instrument in MR analysis. The F-statistic should ideally be greater than 10 in order for an instrument to be considered strong enough to use (221). The F-statistic can be calculated using the following formula:

$$F = \frac{R^2 (n - 1 - k)}{(1 - R^2) k},$$

where k is the number of instruments (or variables) and n is the sample size (Rice 1995). The F-statistic was calculated from the average of the weighted adjusted R^2 in each study.

Mendel's law (222) implies that the genetic variant will be independent of any confounding factors. Therefore, the effect of the genetic variant association with 25(OH)D when adjusted for the social, lifestyle and dietary covariates that could potentially confound the relationship between 25(OH)D and cognitive function is examined using linear regression models. Logistic regression

models are used to examine the direct association of the genetic variant with the dichotomised social, lifestyle and dietary variables. These models were adjusted for 25(OH)D with the assumption that controlling for the modifiable exposure should not affect the association if pleiotropy exists. The genetic variant associations with the potential confounding factors were also corrected for multiple testing.

Meta-analyses

Meta-analyses were conducted in order to increase power of the MR analyses. In a meta-analysis, the effect size for each study is calculated. The consistency between each individual effect size is assessed to obtain a summary score (223). In order to calculate this summary effect, each study included in the meta-analysis is assigned a weight which is related to its precision. Studies with a larger sample size tend to be more precise and hence are assigned a larger weight (223).

A meta-analysis can be conducted in two ways (224). The first is a fixed-effect meta-analysis. This model assumes there is one true effect size underlying all the studies in the analysis and the difference observed between each individual study is due to sampling error. A random effects meta-analysis assumes that the true effect may vary from study to study as the sample populations differ for example, when the participants differ in age (224).

Heterogeneity is the term used to describe the difference between each individual effect sizes between included studies. While variation can be due to random error or be indicative of an underlying true difference between the studies, heterogeneity refers to the variation in true effect sizes (223). The statistical significance of heterogeneity between the studies can be tested using the Q statistic. The I^2 is a descriptive statistic that reflects the proportion of observed variance. If the I^2 is near zero, then almost all observed variance is spurious, if the I^2 is large, then reasons for heterogeneity should be investigated. If the Q statistic indicated significant heterogeneity at the alpha level of 0.05, a random effects meta-analysis was run, otherwise fixed models

were used. Sensitivity analyses conducted in **Chapter 8**, compared models using both random and fixed effects meta-analysis.

Meta-regression was used to determine the sources of heterogeneity among the different study cohorts included in the observational meta-analyses of **Chapter 8** (225). The potential source of heterogeneity for inclusion in meta-regression such as age and country were chosen *a priori*.

3.5 Summary

- Observational and genetic epidemiology are the two approaches that will be used
- Observational epidemiology can give insight into associations between environmental exposures and health outcomes however reverse causality and confounding can be problematic
- Exploration of gene-environment interactions can help to disentangle observed associations between an environmental exposure and health outcome and assess how associations are affected by genotype
- Mendelian randomisation is an approach that utilises a genetic variant, related to an environmental exposure as a proxy to estimate the causal association between an environmental exposure and health outcome
- The use of both observational and genetic epidemiological approaches will enable the development of a hierarchy of evidence from cross-sectional observational studies that can describe evidence of a relationship, to a Mendelian randomisation analysis that has the potential to reflect causal associations. Using these various methods enables triangulation of results to increase confidence in the findings

Chapter 4 Data

4.1 Introduction

Data for the thesis were collected using participants from cohort studies. These cohort studies involve a group of people who share a common condition or experience, for example, all born in the same year. Many cohort studies collect longitudinal data on a variety of topics including health, social and economic affairs. This study design was chosen based on its ability to contribute rich, detailed information to address the aims of the thesis.

Data were predominately collected using participants from the 1958 British birth cohort (1958BC). Data from eight additional cohorts were used to increase power of genetic epidemiology analyses as discussed in **Chapter 3.** Ethical approval for the 1958BC was outlined in the acknowledgments. All participants from the other cohorts provided informed consent, and ethical approval was granted by local research ethics committees.

During this chapter, I will describe participants of the 1958BC, the measures of exposure and outcomes used and discuss the genetic variants applied. The final section will give an overview of the participants and variables of the additional cohorts that were used.

4.2 1958 British birth cohort

4.2.1 Participants

The 1958BC, also known as the National Child Development Study (NCDS), is an on-going, multidisciplinary study. The 1958BC is the second oldest nationwide cohort after the 1946 National Survey of Health and Development[3] The aim of the 1958BC is to monitor social, behavioural, educational and physical outcomes as well as to collect information regarding economic circumstances, employment and health behaviour (226).

[3] http://www.nshd.mrc.ac.uk/

The 1958BC originally began as the Perinatal Mortality Study (PMS). This study aimed to investigate still-birth and infant mortality. Mothers of babies born in one week in March 1958 in England, Scotland and Wales (approximately 17,415) were interviewed by mid-wives, who completed questionnaires with reference to medical records (227). To date, the cohort has been followed up ten times; at ages 7, 11, 16, 23, 33, 42, 46, 50, including a biomedical survey at 45 years and NCDS9 which began in the summer of 2013 (55 years). However, data from NCDS9 was not available at the time of this thesis. **Table 4.1** gives the target and achieved sample at each sweep in addition to the main sources of information at each time point (228).

During the childhood sweeps at NCDS1-3, immigrants that were born during the survey week in March 1958 were added to the study (*n*=920) (227, 229). Individuals who participated in any of the childhood sweeps were contacted and followed up for NCDS4-8 (229). Deaths were confirmed through receipt of death certification or notification to the study team (216). At the 45 year survey, ethical approval was obtained from the South East Multi-centre Research Ethics Committee (ref. 01/1/44). During the biomedical survey, self-completed questionnaires, blood and saliva samples were administered and a home interview was conducted by a research nurse.

Data collected in the 1958BC has led to a multitude of studies describing the health and socioeconomic characteristics of the participants. The longitudinal design facilities investigation of disease progression and enables the identification of potential risk factors. Some selected findings from the 1958BC include; the effect of family, social circumstances and smoking on perinatal mortality (230), that children of obese and overweight parents have a higher risk of obesity (231) and that children's relationship with both their mother and father predicted mental health problems in adulthood (71). The 1958BC can be compared with earlier and later born cohorts to examine changing patterns of diseases. For example, one study comparing the 1958BC with the 1946 birth cohort found that individuals born in 1958 were no heavier in childhood than those born in 1946 but they were taller and grew faster (232). Furthermore, by mid adulthood, participants in the 1958BC had a higher BMI and higher prevalence of obesity compared with the older cohort (232).

Table 4.1: 1958BC sweep information

	PMS	**NCDS1**	**NCDS2**	**NCDS3**	**NCDS4**	**NCDS5**	**NCDS6**	**NCDS7**	**Biomedical**	**NCDS8**
	1958	1965	1969	1974	1981	1991	2000	2004	2003	2008
Age (y)	Birth	7	11	16	23	33	42	46	45	50
Source	Parents Medical	Parents School Tests Medical	Parents School Tests Medical	Parents School Tests Medical Subject Census	Subject Census	Subject Partner Children	Subject	Subject	Medical Subject	Subject
Target (n)	17,638	17,370	16,880	16,929	16,713	16,389	16,194	16,072	11,971	16,014
Achieved (n)	17,415	15,425	15,337	14,654	12,537	11,469	11,419	9,534	9,377	9,790

In order to generalise findings from the 1958BC to the current British population, the study sample needs to be representative. As with all cohort studies, sample attrition has occurred in the 1958BC. For example, those with a lower social class at birth, lower mathematics score at 7 years and with internalising and externalising behaviours at 7 years were found to be under-represented in the 45 year survey (**Chapter 3**) (216). Although immigrants were included during the childhood studies, the majority of participants of the 1958BC are from a white European population, which was representative of the population at the time (216). Therefore, the 1958BC does not capture the ethnic diversity of today's society. Despite this potential bias, a study examining participants of the 33 year (NCDS5) survey found that they are broadly representative of the general white British population in terms of several socioeconomic characteristics (233). Similarly, participants of the 45 year biomedical survey were shown to broadly represent those born in Britain in 1958 and resemble the white British population (216).

4.2.2 Exposure

Serum vitamin D, or 25-hydroxyvitamin D (25(OH)D) is the main exposure variable used. Information regarding the background, intake, metabolism and thresholds of 25(OH)D is detailed in **Chapter 1**.

Non-fasting blood samples were collected from 88% of participants in the 1958BC during the biomedical survey. 97% of these consented to the creation of immortalised cell lines (234). The venous blood sample was obtained during the 45 year biomedical survey. These blood samples were collected into Sarstedt evacuated tubes without anticoagulant and sent by regular mail to the central laboratory (235). 25(OH)D assessment was performed on *n*=7,591 participants in the Royal Victoria Infirmary (Newcastle upon Tyne, Tyne Hospitals National Health Service Trust, Newcastle upon Tyne UK) (235). An automated application of Immunodiagnostic Systems Ltd. (IDS) enzyme immunoassay (EIA) was used to obtain serum 25(OH)D measurements. The application was evaluated in the central laboratory on a BEP2000 programmable microtiter plate analyser (Dade-Behring, Marburg, Germany)

with sensitivity of 5.0nmol/l, linearity ≤155nmol/l and intra-assay Coefficient of Variation (CV) 5.5-7.2% (235).

Variations between 25(OH)D assays reduces between-study comparisons. Due to problems relating to these variations, there was a need for a rigorous internal quality-assurance scheme for 25(OH)D assays that led to the establishment of the International Vitamin D External Quality Assessment Scheme (DEQAS) in 1989 (236). The primary aim of DEQAS is to

"monitor the performance of individual laboratories…also used to assess the performance of the methods used" (236)

Laboratories that participate in DEQAS are sent five quarterly samples of human serum. These samples are analysed to give an All-Laboratory Trimmed Mean (ALTM). Laboratories receive a certificate if 80% of these results are within 30% of the DEQAS ALTM (236). The use of the ALTM has been criticised. The ALTM has been used as a good surrogate for values obtained by the gas chromatography-mass spectrometry method (237). However, as more methods are being used and developed, ALTM can no longer be regarded as the true value. In this instance, while passing the DEQAS may mean the method is relatively accurate compared with other laboratories, it may not represent the true value of 25(OH)D. With this in mind, more procedures to improve assay comparability are being explored. In 2010, a serum based reference material was proposed and it is hoped that this can be used in the future to produce valid 25(OH)D measurements (238).

25(OH)D measurements in the 1958BC were standardised according to the DEQAS reference (235).

4.2.3 Outcomes

Common mental disorders (CMD)

CMDs were assessed in the 45 year biomedical survey and the 50 year sweep of the 1958BC. The Clinical Interview Schedule Revised (CIS-R) used to assess CMDs at 45 years (239, 240) was administered by a survey nurse using

computer assisted personal interviewing (CAPI). The CIS-R is a standardised semi-structured questionnaire, assessing symptoms of CMDs in the previous week. The CIS-R usually contains 14 sections, however only 9 were administered at 45 years (listed in **Table 4.2**) due to time constraints (241).

Each section of the CIS-R assessed a group of symptoms related to a CMD (see **Table 4.3**). For the purposes of this thesis, depressive, anxiety, panic and phobia symptoms were examined. Filter questions were used to establish the presence of symptoms of a particular CMD in the past month. Positive response to these led to further questions based on symptoms in the past week. For each symptom, scores range from 0 to 4, with a higher score indicating more frequent and severe symptoms. Symptom scores were computed for each disorder. To create a binary variable for each CMD, a score of ≥2 on each scale was defined as clinically relevant, where ≥2 indicated presence of the CMD and <2 symptoms indicated no CMD (98).

Good reliability and validity for the CIS-R has been reported (240). Standardisation aims to transform clinical judgements of presence of CMDs into rules. Therefore, any systematic bias that may present due to between-observer variation of CMDs is reduced by the standardisation of the CIS-R questionnaire. Furthermore, the validity of the results from the CIS-R increased through limiting assessment to symptoms in the previous week with the assumption that recall for psychological symptoms become weaker over a longer period. Two reliability studies conducted in London, UK and Santiago, Chile reported no difference in the CIS-R results between lay interviews and trained psychiatrists (240). The CIS-R has been also used in the Adult Psychiatric Morbidity Survey in England (9) and the EMPIRIC study (242).

Table 4.2: Symptoms of CMDs measured by CIS-R at 45 years in 1958BC

Included in biomedical survey	**Not included in biomedical survey**
Depression	Worry
Anxiety	Obsessions
Panic	Somatic symptoms
Phobias	Compulsions
Depressive ideas	Worry about physical health
Fatigue	
Sleep problems	
Irritability	
Concentration/forgetfulness	

Table 4.3: CMD symptoms assessed using CIS-R at 45 years in 1958BC

Depressive symptoms
Have you had a spell of feeling depressed in the past month
Have you been able to enjoy things as much as you usually do
In the past week have you felt depressed
In the past week have you been able to enjoy things as much as usual
On how many days in the past week have you felt depressed or unable to take an interest in things
Have you felt depressed/unable to take interest in things for more than 3 hours in total on any day in the past week
What sort of things made you feel depressed in the past week
In the past week when you were depressed did you become happier when something nice happened
How long have you been feeling depressed
Anxiety symptoms
Have you been feeling anxious or nervous in the past month
In the past month did you ever find your muscles felt tense
Have you felt anxious when there was no real danger
Was this brought on by a phobia about some specific situation or did you feel generally anxious
On how many of the past 7 days have you felt generally anxious
In the past week how unpleasant has your anxiety been
When you've been anxious have you had any of the symptoms shown on this card & which of these symptoms have you had
Have you felt anxious for more than 3 hours in any day in the past week
How long have you had these feelings of general anxiety
Panic symptoms
In past month did your anxiety get so bad that you thought you might collapse or lose control
How often has this happened in the last week
In the past week, how unpleasant have these feelings of panic been
Did this panic last for longer than 10 minutes
Are you relatively free of anxiety between these panics
Is the panic always brought on by same thing
How long have you been having these feelings of panic
Phobia symptoms
Have you avoided anything because it would have made you feel nervous or anxious even though there was no real danger
Which of these situations made you the most anxious in the past month
Which of these situations did you most avoid in the past month
In the past week how many times have you felt anxious about this situation/thing
In the past week on those occasions when you felt anxious did you have any of these symptoms
Which of these symptoms did you have
In the past week, have you avoided any situation because it would have made you feel anxious, even though there was no real danger
How many times have you avoided such situations in the past seven days
How long have you been having these feelings about these situations

During the 50 year survey, the Mental Health Inventory (MHI-5) was used to assess the presence of depressive symptoms in the past four weeks (versus CIS-R, which examines symptoms in the previous week) (243, 244). Participants completed the MHI-5 as part of a 16-page paper self-completion questionnaire which was mailed to them in advance of their interview (245). **Box 4.1** illustrates the questions used. Each item on the MHI-5 was scored on a 6-point scale ranging from '*all of the time*' to '*none of the time*'. To create an overall score, the responses to each item were summed. Some of the items were reversed before summing to ensure that a high score indicated better mental health for example, item two, '*felt calm and peaceful'.* The scores were then linearly transformed to a 0-100 scale with lower scores indicating worse mental health (246). While MHI-5 was designed as a general mental health measure, several studies have shown that it is most appropriate for measuring depressive symptoms using a cut-off point of ≤52 (247-252).

Box 4.1: Mental Health Inventory-5 questions

How much of the time during the last month have you:

1. been very nervous person
2. felt calm and peaceful
3. felt downhearted and low
4. been a happy person
5. felt so down in the dumps that nothing cheers you up

This short version of the MHI was compared with the 18-item MHI and the 30-item version of the General Health Questionnaire (GHQ-30) and a 28-item Somatic Symptom Inventory (SSI-28). The MHI-5 was found to be as good as the MHI-18 and the GHQ-30 and better than the SSI-28 at detecting major depression, affective disorders and anxiety disorders (based on Diagnostic Interview Schedule diagnosis) (244). The MHI-5 has been used in the past to measure depressive symptoms in other population studies, for example, the Nurse's Health study (249).

Cognitive function

In the 50 year survey of the 1958BC, cognitive function was assessed during a 60 minute face to face interview in the participant's home. The test consisted of four memory and concentration tasks, where each task measured different aspects of cognitive function. **Figure 4.1** shows the domains assessed. The interviewers also recorded any factors that might have influenced performance on cognitive tests (**Table.4.4.**) The immediate word recall test examined how many words a participant could immediately recall from a list of 10 common words (**Table 4.5**.). Participants had up to two minutes to recall as many words as possible.

The delayed word list examined how many of these words the participants could recall after an interval of approximately 5 minutes, during which the other cognitive tests were conducted. There were four word lists which were randomly assigned during these tasks (**Table 4.5**). Additionally, while the lists were mainly read out by a computer voice; in 2% of cases the interviewer read out the lists at an equivalent pace to the computer.

The animal naming task was used to assess verbal fluency. This test measured how quickly the participants could name as many different words from a particular category, in this case, animals. Participants had one minute to compete this task. Repetitions, named animals (for example, Dumbo) and redundancies (for example, white cat, black cat) were excluded from the total score. This test version was taken from the cognitive assessment section of the Cambridge Mental Disorders of the Elderly Examination (CAMDEX) (253).

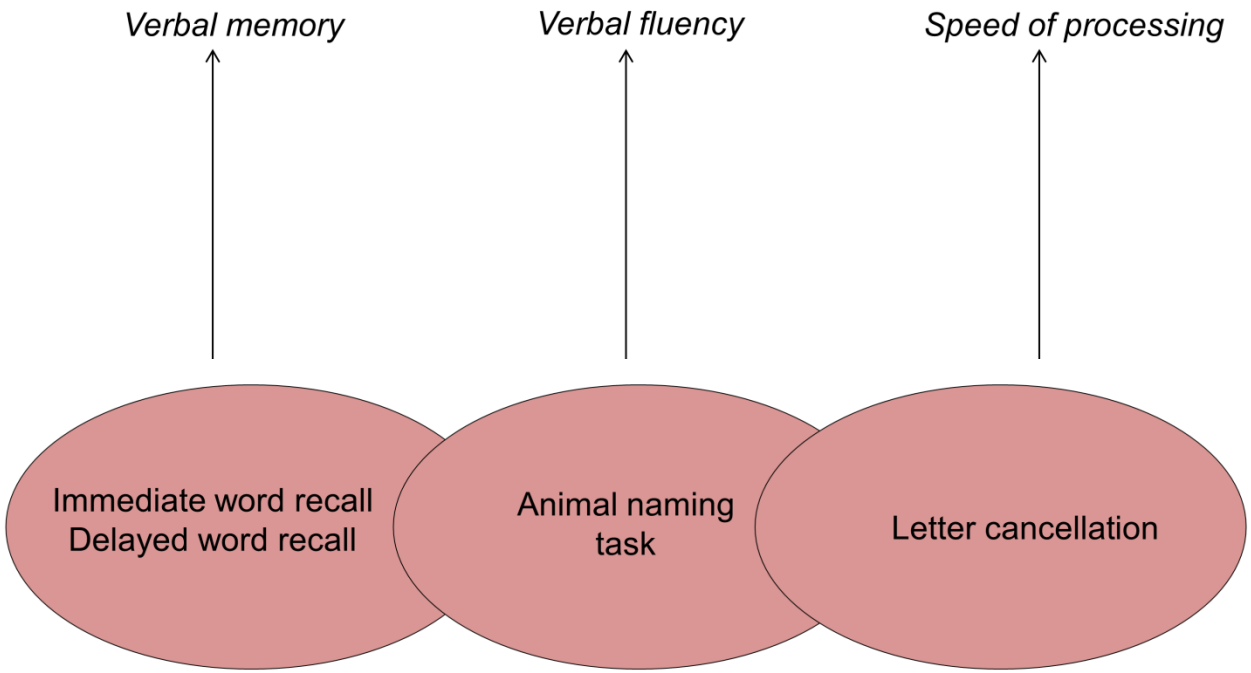

Figure 4.1: Cognitive tests conducted at 50 years in 1958BC

Table 4.4: Factors affecting cognitive tests at 50 years in 1958BC

Cognitive testing conditions	**Description**
Day and time of cognitive testing	Weekday morning, weekday afternoon, weekday evening, weekend morning, weekend afternoon, or weekend evening
Presence of others in the room	yes, no
Word list used*	a, b, c, d
Administration of word list	computer or interviewer
Other contextual factors affecting performance[†]	yes, no

*Only relevant for immediate and delayed word recall test

[†]Includes: blind or poor eyesight; deaf or hard of hearing; too tired; Illness or physical impairment that affects the ability to perform; impaired concentration; very nervous or anxious; mental impairment; interruption or distraction; noisy environment; problems with the laptop; difficulty understanding English

Table 4.5: Word lists for cognitive tests at 50 years in 1958BC

Word list A	**Word list B**	**Word list C**	**Word list D**
Hotel	Sky	Woman	Water
River	Ocean	Rock	Church
Tree	Flag	Blood	Doctor
Skin	Dollar	Corner	Palace
Gold	Wife	Shoes	Fire
Market	Machine	Letter	Garden
Paper	Home	Girl	Sea
Child	Earth	House	Village
King	College	Valley	Baby
Book	Butter	Engine	Table

Finally, the letter cancellation task (developed for the 1946 birth cohort (254)) assessed mental speed, attention and visual scanning. This task involved a page with 125 upper case letters of the alphabet arranged randomly in 26 rows and 39 columns. Participants were instructed to cross out as many target letters (P and W, 65 in total) as possible in one minute. Participants are instructed to work across each row from left-to-right. When the time is up, participants were asked to underline the last letter they reached. The total number of letters searched i.e. the sum of all the items processed, regardless of accuracy, provided an assessment of processing speed.

These cognitive measures have been used in other longitudinal studies (for example, the 1946 British birth cohort (255) and the English Longitudinal study of Ageing (256)). In order to increase comparability of the cognitive tests both within the 1958BC and with other studies, cognitive scores were standardised to z-scores whereby the mean was 0 and standard deviation was 1 using the following formula:

$$Z = \frac{X - \mu}{\sigma},$$

where z represents the standardised cognitive score, X is the unstandardized cognitive score, μ is the mean and σ is the standard deviation.

4.2.4 Covariates

Many demographic and lifestyle characteristics from the 1958BC were used. During this section I will give a brief overview of covariates. The categorisation of each covariate will be described in relevant chapters.

Demographic

Ethnicity was self-reported at 42 years and grouped into two broad categories i.e. white and other, due to the small number of other ethnic groups in the cohort. Region of residence was self-reported during the 46 year survey using computer assisted telephone interviewing. Regions included North, Yorkshire and Humberside, East Midlands, East Anglia, south East, South West, West Midlands, North West, Wales, Scotland and Isle of Man and Channel Isles.

Socioeconomic position (SEP) in adulthood was assessed at 42 years (NCDS5) and defined using the Registrar General's classification (257). Educational attainment of cohort members was based on the highest qualification obtained by 42 years, or by 33 years if data was missing.

Early life factors

SEP in childhood was based on father's occupation at the participant's birth (or at 7 years if this data was missing) and categorised using the Registrar General's classification (257). Cognitive ability in childhood was assessed at 7, 11 and 16 years from tests administered at school. Mathematics ability at 7 years was assessed using 10 problems with increasing levels of difficulty (range 0-10) (258). Arithmetic tests at 11 years (range 0-40) and 16 years (range 0-31) were constructed specifically for use in the 1958 cohort by the National Foundation for Educational Research in England and Wales and Manchester University respectively. Reading ability was assessed using the Southgate test at 7 years (range 0-30) (259) and an assessment similar to the Watts Vernon comprehension tests was constructed by the National Foundation for Educational Research in England and Wales at 11 and 16 years (range 0-35). A standardised test for general ability, consisting of verbal (range 0-40) and non-verbal components (range 0-40), was conducted when participants were 11 years (260). Pearson correlations between childhood tests ranged from 0.44 to 0.78 (p<0.001 for all). Tests were standardised for age at assessment to control for potential age-effects of cognitive ability, and averaged to obtain a summary score for childhood cognitive ability (261). Missing data on one test were replaced by that individual's mean standardised score on the other tests. Internalising and externalising behaviour was assessed at ages 7, 11 and 16 years. At 7 and 11 years, these behaviours were measured using the teacher-rated Bristol Social Adjustment Guides which assess 12 different syndromes using 146 items (262). Depression and withdrawal and were classified as internalising problems while anxiety, hostility and restlessness were indicative of externalising problems (69). At 16 years, internalising and externalising problems were measured using the teacher version of the Rutter scales (263).

Physical status and lifestyles

Menopausal status i.e. post-menopausal, pre-menopausal or peri-menopausal was obtained from questions regarding menstruation at 45 years during the biomedical survey. Smoking and alcohol consumption were self-reported at ages 42 and 45 years respectively. BMI was defined using the participants weight in kilograms divided by height measured in meters squared (kg/m^2). Weight was measured using Tanita solar scales and height was measured with participants in light clothes and without shoes taken by a nurse at the participant's home. Frequency of physical activity was reported at 42 years.

Vitamin D-related lifestyles

Vitamin D-related lifestyles were all self-reported at 45 years (26). These included the frequency of consumption of oily fish (such as salmon, trout, mackerel, sardines or fresh tuna), margarine and use of supplements containing cod liver, fish oil or vitamin D. The amount of time spent outside during the past month and leisure time spent using the television (TV) or personal computer (PC) was also reported along with the frequency of suncover usage, blistering after sunburn, and seeking suntan. Use of other dietary supplements i.e. pills powders, tablets or drops were reported at 45 years.

4.2.5 Genetic data

Information regarding the apolipoprotein E (*APOE*) gene and genetic variants related to 25(OH)D are required for **Chapters 7** and **8**. 8,018 participants from the 1958BC biomedical survey gave consent for DNA to be extracted from their blood samples. The genetic variants used are single nucleotide polymorphisms (SNPs) (**Box 4.2**). A glossary of genetic terms can be found in **Appendix 2**.**1**. The *APOE* and vitamin D-related SNPs are discussed separately below.

Box 4.2 Definition of single nucleotide polymorphism (SNP)

A SNP refers to a sequence of DNA within a gene that differs between individuals (3). Specifically, variation occurs when a single nucleotide i.e. adenine (A), guanine (G), thymine (T) or cytosine (C) differs between individuals. Therefore, there is potentially more than one form of the gene consisting of a number of *alleles* (see **Appendix 2.1**).

Apolipoprotein E

APOE is a polymorphic gene consisting of three major alleles formed from two SNPs i.e. rs429358 and rs7412. The SNPs possess an amino acid substitution of arginine to cysteine at positions 112 and 158 of the apolipoprotein E protein (264). The common allelic forms are ε2, ε3 and ε4. Each individual possesses two *APOE* alleles, inheriting one from each parent. Therefore, there are six possible *APOE* genotypes: ε2/ε2, ε2/ε3 ε2/ε4 ε3/ε3 ε3/ε4 and ε4/ε4. The *APOE* genotypes have varying frequencies in human populations. *APOE* ε3 is the most commonly occurring form worldwide followed by ε4 (**Table 4.6**).

Table 4.6: *APOE* allelic variation and worldwide frequencies (40)

	Nucleotide variation		**Amino acid variation**		**Allele frequency***
	rs7412	rs429358	158	112	
APOE ε2	T	T	Arginine	Arginine	0 - 0.15
APOE ε3	C	T	Cysteine	Arginine	0.55 - 0.91
APOE ε4	C	C	Cysteine	Cysteine	0.05 - 0.41

*Data from (264)

In the 1958BC, *APOE* genotypes were obtained from the Illumina iSelect Metabochip genotyping array (265) from those who consented to genotyping during the biomedical survey (*n*=5,550). The Metabochip is a custom genotyping array that can assay nearly 200,000 SNP markers (265).

Vitamin D-related genetic variants

As mentioned in **Chapter 1**, previous research has suggested that there is a genetic influence on vitamin D status, with heritability estimates ranging from 29 to 80% (37-39). The identification of appropriate genetic proxies for 25(OH)D is essential for an MR study (3).

The selection of vitamin D-related SNPs for **Chapter 8** came primarily from two Genome-Wide Association (GWA) studies (5, 6). One was carried out in the USA using 4,501 participants in the discovery phase and 2,221 in the replication analysis (6). The other was carried out by SUNLIGHT consortium cohorts (Study of Underlying Genetic Determinants of Vitamin D and Highly Related Traits), which included participants from the 1958BC. GWAS using the SUNLIGHT consortium consisted of 16,125 participants in the discovery stage and 17,871 in replication (5). Both studies used participants from European ancestry. Both studies identified three SNPs near genes involved in the metabolism of vitamin D i.e. 7-dehydrocholesterol reductase (*DHCR7,* 7-dehydrocholesterol reductase), 25-hydroxylase (*CYP2R1,* cytochrome P450, family 2, subfamily R, polypeptide 1), vitamin D-binding protein (VDBP) (*GC*, group-specific component). The SUNLIGHT meta-analysis also identified variants affecting the clearance of 25(OH)D i.e. *CYP24A1* (cytochrome P450, family 24, subfamily A, polypeptide 1) (**Figure 4.2** and **Box 4.3**).

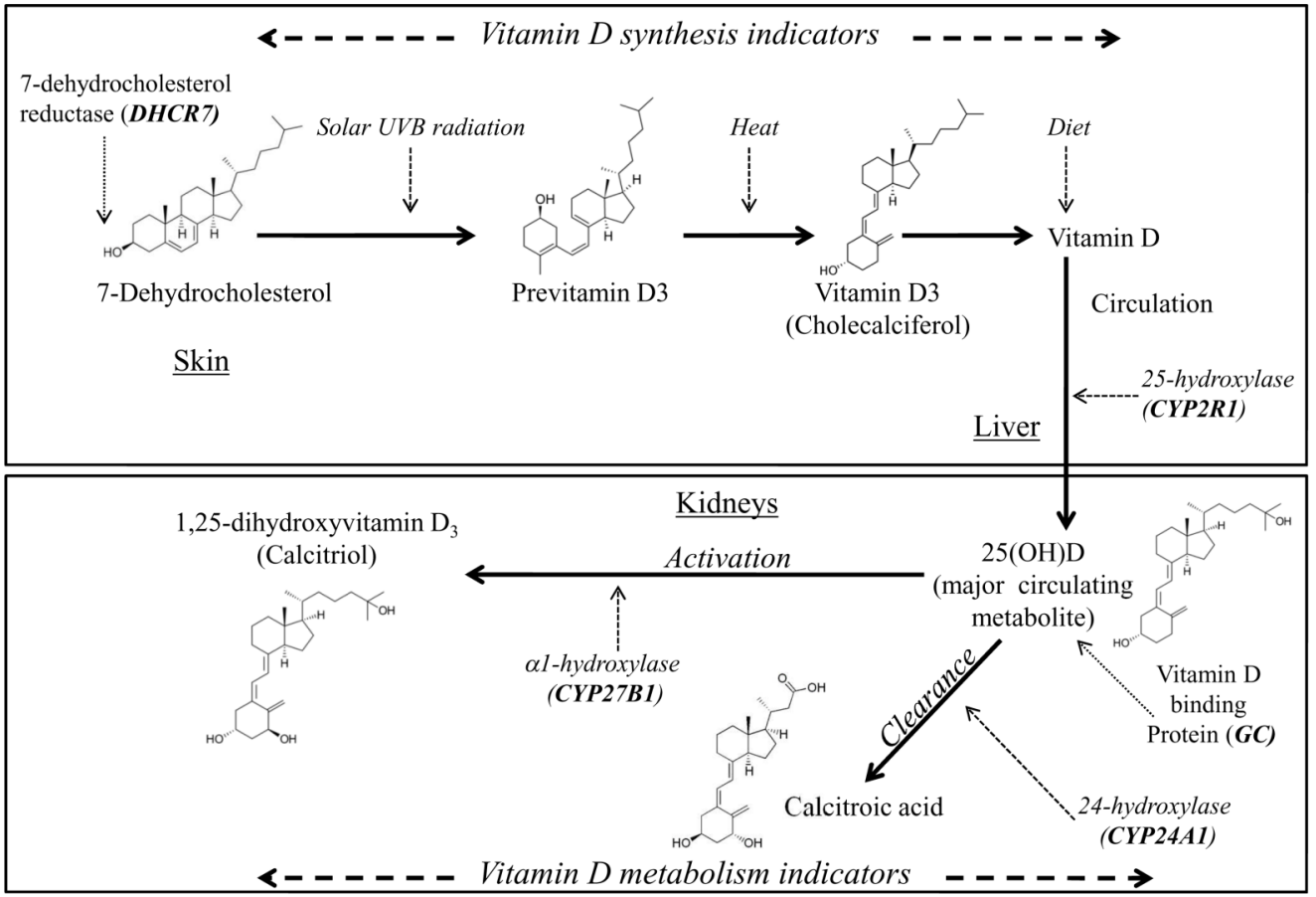

Figure 4.2: Genetic variants on the vitamin D metabolic pathway

Taken from Berry, 2012 (207)

Box 4.3: 25(OH)D-related SNPs

- *DHCR7* produces the enzyme that catalyses the conversion of 7-dehydrocholesterol in the skin to cholesterol. This will result in the removal of pre-cholesterol from the vitamin D metabolism pathway, thus reducing the production of 25(OH)D (4).
- *CYP2R1* is involved in the hydroxylation of vitamin D by 25-hydroxylase to produce 25(OH)D. This gene was one of the top hits in both GWAS (5, 6).
- *GC* codes VDBP which binds to 25(OH)D and transports it to target organs *(7)*
- *CYP24A1* catabolises 25(OH)D via the 24-hydroxylase into the water soluble calcitroic acid which is excreted in the bile *(8)*

Further work conducted in 2012 confirmed that these SNPs are appropriate instruments for MR studies using data from 6,877 participants from the 1958BC (207). Additionally, *DHCR7* and *CYP2R1* have been used in a previous MR analysis to proxy 25(OH)D concentrations (33).

There is evidence to suggest that VDBP (coded by *GC*) may affect the bioavailability of 25(OH)D (266). *CYP24A1* is regulated by other hormones, for example, parathyroid hormone. Therefore the extent to which *GC* and *CYP24A1* can proxy 25(OH)D concentrations is unclear. Thus, *DHCR7* and *CYP2R1* will be used for MR analysis in **Chapter 8**.

Genotyping participants for *DHCR7* (rs12785878) and *CYP2R1* (rs12794714) in the 1958BC came from two main resources utilising the 1958BC as sub-samples for control populations in genome-wide studies:

1) *Wellcome Trust Case Control Consortium (WTCCC) (267)*

The WTCCC genotyped 1,500 randomly selected participants of the 1958BC biomedical survey who had consented for DNA samples to be taken. Genotyping was conducted on Affymetrix Genechip 500K and Illumina Infinium 550K chip (267). During a second phase of the WTCCC (WTCCC 2), these subjects were re-genotyped along with an additional 1,500 individuals (*n*=3000) using the Affymetrix 6.0 and Illumina 1 (268). The genotyping calling algorithm was Chiamo (269). Information from WTCCC 2 was used.

In order to insure quality, genetically similar samples, those from non-European ancestry and those exceeding the heterozygosity thresholds were excluded. Further samples were excluded i.e. those with outlying allele intensities from a selection of SNPs compared with the larger sample, those with discordance with external genotypes and samples with gender discrepancy. Definition of quality measures can be found in **Appendix 2.1**. The minor allele frequency (MAF) refers to the frequency of the least common allele of the gene in a given population. SNPs that had a MAF of <1% were excluded so that SNP differences in the sample could be captured. Call rate indicates the amount of genotypes that were missing for each individual. Individuals with a call rate of <0.97% were excluded to ensure good quality genotyping in the sample. The Hardy-Weinberg equilibrium (HWE) tests if the alleles segregate randomly in the population following expected genotype frequencies. Samples with a HWE p-value < 1e-20 were excluded.

2) *Type 1 Diabetes Genetics Consortium (T1DGC) (270)*

The T1DGC genotyped 2,596 randomly selected, consenting participants of the 1958BC biomedical survey, exclusive of the WTCCC subjects. Genotyping was conducted using the Illumina Infinium 550K (270). The genotyping calling algorithm was Illuminus (271).

Samples that exceeded the call rate of 3%, exceeded the heterozygosity thresholds, had non-European ancestry and gender discrepancy were

excluded. SNPs that had MAF <1% and HWE p-value <1e-7 were also excluded.

The quality control measures aim to highlight potential issues with genotyping, duplicates, related individuals, and contaminated samples in both WTCCC2 and T1DGG and exclude individuals that don't meet the standards (272). Work conducted in a PhD thesis in 2012 provided further assurance of the suitability of genetic data from WTCCC2 and T1DGG for use in genetic studies (273).

4.3 Additional cohorts

As mentioned in the introduction, eight additional cohorts contributed data for **Chapter 8**. One additional cohort (i.e. PIVUS) had information on *APOE* genotype and memory function, therefore this cohort was included in **Chapter 7**. Additional cohorts include:

1) Austria Stroke Prevention Survey (ASPS)
2) English Longitudinal Study of Ageing (ELSA)
3) Epidemiologische Studie zu Chancen der Verhütung, Früherkennung und optimierten Therapie chronischer Erkrankungen in der älteren Bevölkerung (ESTHER)
4) Helsinki Birth Cohort Study (HBCS)
5) The Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) – also contributing to **Chapter 7**
6) The Tromsø Study (Tromsø)
7) Uppsala Longitudinal Study of Adult Men (ULSAM)
8) Whitehall II (WHII)

Further details on the participants, exposure, outcome and genotyping are given in the remainder of this chapter. Also presented is a comparison of the 1958BC with the eight additional cohorts.

ASPS

Participants

The ASPS is a prospective study on the effects of vascular risk factors on brain structure and function in cognitively normal inhabitants of Graz, Austria (274, 275). 2,007 participants aged 45 to 86 years were recruited from the official community register and all participants were free of stroke and dementia. Individuals were excluded from the study if they had a history of neuropsychiatric disease. Between 1991 and 1994, 509 participants randomly selected from the entire cohort underwent cognitive testing. To enlarge the cohort with imaging and neuropsychological assessments, an additional 567 individuals were randomly selected in a second study between 1999 and 2003. Participants of the first and second panels were pooled, which resulted in a total of 1,076 individuals with blood and cognitive tests. 1,003 participants had information on at least one cognitive test and 829 participants had genotype information.

Exposure

Participants from this cohort did not have data on 25(OH)D concentrations.

Outcome

- *Word association*: Assessed during Bäumler's Lern-und Gedächtnistest (LGT-3) (275) to measure verbal memory
- *Digit association*: Assessed during LGT-3 to measure verbal memory
- *Story recall*: Assessed during LGT-3 to measure verbal memory
- *Trail recall*: Assessed during LGT-3 to measure visuospatial memory
- *Design recall*: Assessed during LGT-3 to measure visuospatial memory
- *Wisconsin Card Sorting test*: Used as a measure of conceptional reasoning.
- *The Alters–Konzentrations–Test*: Used to test attention and speed
- *Trail Making Test B (TMT B):* Participants are asked to draw lines with a pencil between the numbers in the right order as fast as possible. The score is equal to the time in seconds. TMT B consists of digits and letters

1-A-2-B etc. to measure attention, processing speed and executive control

- *Digit span*: Used to test attention and speed
- *Complex reaction time*: Participants react selectively to a specific combination of a visual and acoustic signal by pressing a button as quickly as possible

Genotyping

Genotyping was done at the Human Genotyping Facility, Genetic Laboratory Department of Internal Medicine, Erasmus MC, Rotterdam, The Netherlands, using the Illumina Human610-Quad BeadChip. Participant-specific quality controls included filters for call rate (<98%), and high autosomal heterozygosity (FDR <1%). Quality filters used a call rate >98%, a minor allele frequency >0.01 and a Hardy-Weinberg *p*>1x10-6. ASPS used the Markov Chain Haplotyping (MaCH) package (http://www.sph.umich.edu/csg/abecasis/MACH, version 1.0.15) for imputation to plus strand of NCBI build 36, HapMap release #22.

ELSA

Participants

ELSA is an English based cohort consisting of men and women aged 50 years and over. It is a follow-up study of respondents to the Government's Health Survey for England (HSE)[4], an annual cross-sectional survey designed to be representative of the community-based population in England. The main objective of ELSA is to collect longitudinal multidisciplinary data relating to health, disability, biological markers, well-being, social participation and economics. ELSA began in 2002 and has been followed up every two years. The sample consists of individuals seen in HSE in 1998, 1999 or 2001. 11,391 participants were interviewed for wave 1 (2002) in which cognitive testing was conducted (276). 6,219 participants had information on at least one cognitive test from wave 1. 8,780 participants were interviewed in wave 2 (2004), in

[4] http://www.dh.gov.uk/

which blood samples were taken for DNA analysis (276). 5,617 participants had genotype information.

Exposure

Participants from this cohort did not have data on 25(OH)D concentrations.

Outcome

- *Immediate word recall* (277): Recall as many words as possible from a list of 10 common words read out by interviewer
- *Delayed word recall* (277): Recall as many words as possible from a list of 10 common words read out by interviewer, following a short delay of approximately 5 minutes
- *Verbal fluency, animal naming* (278): Measures how many words related to a category (in this case, types of animals) a participant can produce. Used to measure recall and executive control
- *Letter cancellation* (254): Measures speed of processing. Participants were instructed to cross out as many target letters (P and W, 65 in total) as possible in one minute. Used to measure speed of processing

Genotyping

DNA was extracted from blood samples using magnetic bead technology (Medical Solutions, Nottingham). Genotyping was performed using the KASPar methodology. The call rates and the concordance rates of the four SNPs were >98%, HWE values were $p>0.08$.

ESTHER

Participants

ESTHER is a population-based cohort study conducted in Saarland, Germany. The main aim of ESTHER is to contribute information for the prevention, early detection and treatment of chronic diseases. A detailed characterization of this ongoing cohort study and the study population has been provided earlier (279, 280). Participants were recruited during health screening visits to their general practitioners. The baseline sample (2000-2002) included 9,949 participants aged between 50 and 74 years, in which blood samples for DNA and 25(OH)D were collected. 8,502 had genotype information and 9,581 had information on 25(OH)D concentrations. 8,271 participants were included in the 5-year follow-up (2005-2008) in which cognitive testing was conducted. 1,697 had information on at least one cognitive test.

Exposure

Baseline serum 25(O)D concentrations were measured with the DiaSorin-Liason (Diasorin, Inc., Stillwater, USA) in 2006 and the IDS-iSYS (Immunodiagnostic Systems GmbH, Frankfurt Main, Germany) immunoassay in 2010 for women and men, respectively. Values for 25(OH)D were standardised with liquid chromatography tandem-mass spectrometry (LC-MS/MS) in the Department of Clinical Chemistry, Canisius Wilhelma Hospital, Nijmegen, The Netherlands (281).

Outcome

The Cognitive Telephone Screening Instrument (COGTEL) (282) was used to assess cognitive function.

- *Verbal short-term memory* (283): Recall from a list of 8 word pairs following a short delay
- *Working memory* (284): In reverse order, recall sequences of single-digit numbers which are presented at a 1 second rate

- *Verbal fluency* (285): Includes two tests, letter fluency i.e. producing as many words as possible beginning with a given letter within 60 seconds and category fluency i.e. producing as many different types of professions as possible within 70 seconds
- *Inductive reasoning* (284): Involves the continuation of a mathematical sequence.
- *Verbal long-term memory* (283): Recall from a list of 8 word pairs following a delay during which the other tests were conducted

Genotyping

Samples were genotyped using the TaqMan OpenArrayTM SNP Genotyping Platform (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. The overall call rate was 97.3%. The genotyping concordance was 100%

HBCS

Participants

The HBCS is a longitudinal study focused on the early origins of health and disease (286). Information for this study was collected using data from the maternity hospital, clinical, school databases and other registries on people born in 1934-1944 in two hospitals in Helsinki Finland. 2,003 men and women participated in the clinical follow-up study (2001-2003) in which blood samples for DNA and 25(OH)D were obtained and cognitive testing was administered. Genetic data is available from 1,728 participants, 1,079 had information on 25(OH)D concentrations and 1,054 participants had information on at least one cognitive test.

Exposure

Serum 25(OH)D concentrations were measured using the Chemiluminescent Microparticle Immuno Assay.

Outcome

The HBCS uses CERAD (The Consortium to Establish a Registry for Alzheimer's disease) which consists of the following tests:

- *Verbal fluency, animal naming*: Measures how many words related to a category (in this case, types of animals) a participant can produce
- *Immediate verbal recall*: Recall as many words as possible from list of 10 words in 90 seconds.
- *Delayed verbal recall*: Recall as many words as possible from list of 10 words after a delay
- *Verbal Learning*: Participant is shown a list of 10 words 3 times then recalls as many words as possible. Total score is the number of correct words recalled.

Genotyping

DNA was extracted from blood samples and genotyping was performed with a modified Illumina 610k chip by the Wellcome Trust Sanger Institute, Cambridge, UK. Genetically related individuals and those with gender discrepancies were excluded. After quality control procedures 1,720 samples remained for the analyses. SNPs were included in the analyses if they met the following conditions: call rate ≥ 0.95, minor allele frequency ≥ 0.01, and HWE test with P ≥1x10-5. Genomic coverage was extended to ~2.5M common SNPs by imputation using the HapMap phase II CEU data (NCBI build 36 (UCSC hg18)) as the reference sample and MACH software. SNPs with low imputation quality (r-squared < 0.30), low minor allele frequency (MAF <0.01), or that diverged from HWE (1x10-5), were excluded from the analyses.

PIVUS

Participants

The PIVUS[5] study includes men and women aged 70 years between 2001 and 2004 in Uppsala, Sweden. The primary aim of this study was to evaluate the usefulness of different measurements of endothelial function and other techniques to evaluate vascular function. The baseline study consisted of 1,016 participants in which blood samples were obtained for DNA and 25(OH)D concentrations. 989 participants had genetic data and 1,002 had information on 25(OH)D concentrations. Cognitive testing was conducted in the 5-year follow-up (2006-2011, *n*=827). 799 individuals had information on at least one cognitive test.

Exposure

Serum 25(OH)D was measured at the department of Clinical Chemistry at Uppsala University Hospital using the DiaSorin-Liason immunoassay. CV for inter-assay analyses is 18.4% at a 25(OH)D level of 39.5 nmol/L and 11.7% at 121.2 nmol/L. The intra-assay CV is 7.1% at 44.7 nmol/L and 3.6% at 120.0 nmol/L.

Outcome

- *Mini Mental State Examination (MMSE)* (287): 30-point questionnaire used to screen for cognitive impairment
- *7 minute Screen test* (288): Consists of (1) Benton temporal orientation i.e. measurement of orientation in time (2) enhanced cued recall i.e. identification and recall of 16 pictures immediately and after an interval with semantic cues if necessary (3) Clock drawing i.e. subject draws the face of a clock and places the hands on a fixed time (4) Verbal fluency i.e. participant names as many different animals as possible in one minute
- *TMT A*
- *TMT B*

[5] http://www.medsci.uu.se/pivus/

Genotyping

SNPs were genotyped as part of a larger study at the SNP technology platform at Uppsala University (http://www.genotyping.se/) on an Illumina BeadStation 500GX using Infinium iSelect and Golden Gate assays from Illumina Inc (289). Genotyping calls were performed with Illumina BeadStudio or GenCall software. Samples with low call rate (<90 %), excess heterozygosity or cryptic relatedness were excluded from analyses. SNPs with a call rate less than 90%, or that failed HWE (exact p-value<1x10-6) were excluded from the study.

Tromsø

Participants

Tromsø is a longitudinal population-based multi-purpose study focusing on lifestyle-related diseases in Norway. It began in 1974 with 6,595 male participants. Blood collection and DNA preparation was conducted in Tromsø 4 (1994-1995, *n*=27,158) (290). 7,161 participants had 25(OH)D measures and 12,029 had genetic data. Cognitive tests were conducted in Tromsø 5 (2001, *n*=8,130). 5,267 individuals completed at least one cognitive test.

Exposure

Serum 25(OH)D concentrations were measured by an electrochemiluminescence immunoassay (ECLIA), using an automated clinical chemistry analyser (Modular E170, Roche Diagnostics®, Mannheim, Germany). The total analytical CV for the 25(OH)D assay was 7.3%.

Outcome

- *Finger tapping test* (291): Participant is asked to tap as many times as possible in 10 seconds with their index finger on a computer
- *Digit symbol coding test* (284): Consists of rows containing blank squares, each paired with a randomly assigned number from one to nine, and a printed key about that pair, each number with a different nonsense symbol. Participants consecutively fill-in as many as possible of the blank spaces with the corresponding symbol as quickly as possible for 90 seconds
- *12 word memory test* (291): Words were shown written on a board and pronounced. Participants had 2 minutes to recall the word

Genotyping

Genotyping was conducted by KBioscience (http://www.kbioscience.co.uk) using KASP (KBioScience Allele-Specific Polymorphism) SNP genotyping system. Two separate manual quality control checks were performed, and the data was also checked by specific software to determine that there are no incorrect call assignments, no samples too close or too far from the origin or any incorrect calls. The call rate for all the 4 SNPs was >98 % and the Hardy-Weinberg equilibrium p value was $p>0.01$.

ULSAM

Participants

ULSAM is a longitudinal study based on all men born between 1920 and 1924 in Uppsala, Sweden. The study began in 1970 when the men were 50 years (292). The main aim of the study was to identify cardiovascular risk factors. Blood collection, DNA preparation and cognitive testing was conducted when participants were age 70 years (1991-1995 $n=1,221$). 1,124 participants had genetic data 1,194 had information on 25(OH)D concentrations and 999 had information on at least one cognitive test.

Exposure

Serum 25(OH)D concentrations were determined with high-pressure liquid chromatography (HPLC) atmospheric pressure chemical ionization (APCI) mass spectrometry (MS) at Vitas, Oslo, Norway (www.vitas.no). HPLC was performed with a Hewlett Packard 1100 liquid chromatography (Agilent Technologies, Palo Alto CA, USA) interfaced by APCI to a Hewlett Packard mass spectrometer operated in single-ion monitoring mode (SIM). Recovery is 95%; the method is linear from 5-400 nmol/L and the limit of detection is 1-4 nmol/L. The CV for inter-assay analyses are 7.6% at 25(OH)D concentrations of 47.8 nmol/L and 6.9% at 83.0 nmol/L. The intra-assay CV was 5.1% at 47.8 nmol/L and 6.1% at 83.0 nmol/L. The assay is accredited by DEQAS.

Outcome

- *MMSE* (287): 30-point questionnaire used to screen for cognitive impairment
- *TMT A* (293)
- *TMT B* (293)

Genotyping

As for PIVUS, SNPs were genotyped as part of a study of SNPs at the SNP technology platform at Uppsala University (http://www.genotyping.se/) on an Illumina BeadStation 500GX using Infinium iSelect and Golden Gate assays from Illumina Inc. Genotyping calls were performed with Illumina BeadStudio or GenCall software. Samples with low call rate (<90 %), excess heterozygosity or cryptic relatedness were excluded from analyses. SNPs with a call rate less than 90%, or that failed (exact P-value<1x10-6) were excluded from the study.

<u>WHII</u>

Participants

WHII was established in 1985 with the aim of investigating the importance of socioeconomic difference in physical, mental illness and mortality in London-

based offices of 20 Civil Service departments (294). DNA preparation and cognitive testing was conducted using information from phase 7 (2002-2004, *n*=6,967). Genetic information was available for 5,067 participants and 6,362 had information on at least one cognitive test.

Exposure

Participants from this cohort did not have data on 25(OH)D concentrations.

Outcome

- *Memory test score* (295): Recall in writing as many of the 20 two syllable words in any order within two minutes
- *Alice-Heim 4* (296): Composed of a series of 65 verbal and mathematical reasoning items of increasing difficulty. It tests inductive reasoning, measuring the ability to identify patterns and infer principles and rules. Participants had 10 minutes to do this section
- *Mill Hill Score* (297): Vocabulary was assessed using the multiple choice format consisting of a list of 33 stimulus words ordered by increasing difficulty and six response choices
- *Verbal fluency (phonemic)* (298): Recall in writing as many words beginning with 's' as they could in one minute
- *Verbal fluency (semantic)* (298): Recall in writing as many animal names as they could in one minute

Genotyping

DNA was extracted from blood samples using magnetic bead technology (Medical Solutions, Nottingham). Genotyping was performed using the Illumina 50K IBC CVD chip or KASPar methodology. The call rates and the concordance rates of the four SNPs were >98%. The HWE p values were $p>0.07$.

Overall, five cohorts had data on the exposure i.e. 25(OH)D concentrations (ESTHER, HBCS, PIVUS, Tromsø, and ULSAM). All cohorts had data on the main outcome i.e. cognitive function. In order to harmonise cognitive tests, each outcome was standardised to produce a mean of zero and a standard deviation of one through the following steps:

1) Standardise each individual cognitive test in each cohort:

$$Z = \frac{X - \mu}{\sigma},$$

where z represents the standardised cognitive score, X is the unstandardised cognitive score, μ is the mean and σ is the standard deviation.

2) Obtain a summary score for both memory domain and global cognitive function:

$$\text{memory or global congition} = \text{z}(test\ 1) + z(test\ 2) + z(test\ 3) + \cdots$$

3) Re-standardise the memory and global cognitive function to produce a mean of zero and standard deviation of one:

$$Z = \frac{X - \mu}{\sigma},$$

In this case z represents the standardised memory or global cognition score, X is the score of the test created in step two, μ is the mean of this score and σ is the standard deviation.

Data on *DHCR7* and *CYP2R1* was available in all cohorts. When the SNPs rs12785878 or rs12794714 were not accessible, proxy SNPs which were in perfect linkage disequilibrium (i.e. SNPs that descend from single ancestral chromosomes and therefore are non-randomly associated) were used.

For genetic analyses, main covariates from additional cohorts included gender, age, month of blood collection, education and depressive symptoms. **Table 4.7**

describes how the main covariates for **Chapter 8,** education and depressive symptoms, were measured in each cohort.

4.4 Comparison of 1958BC with additional cohorts

Table 4.8 compares participant characteristics between the cohorts based on those eligible for analyses in **Chapter 8**. While the 1958BC consists of an approximately equal distribution of men and women, ULSAM comprises an exclusively male population and WHII consists of mostly men (73.9%). All participants from the 1958BC are <65 years while participants from ESTHER, PIVUS and ULSAM are all ≥65 years.

Table 4.7: Description of education and depressive variables in additional cohorts

Cohort	Education		Depressive symptoms	
	Age (years, (SD))	Description	Age (years, (SD))	Description
ASPS	65.6 (8.0)	9, 10, 13 or 18 years in education	65.6 (8.0)	EWL (range 0-15)
ELSA	NA	NA	66.1 (9.7)	8-item CES-D: <3, ≥3
ESTHER	62.1 (6.6)	≤9, 10-11 or ≥12 years in education	74.0 (2.8)	15-item GDS: <5, ≥5
HBCS	68.1 (2.9)	Folk school*/elementary/middle school, learning profession, elementary school or similar or lower or higher university degree	68.1 (2.9)	CES-D: <16, ≥16
PIVUS	70.2 (0.2)	primary school (<9 years), secondary school (9-12 years), university level (>12 years)	NA	NA
Tromsø	64.5 (9.9)	continuous variable measuring years of education	64.5 (9.9)	HSCL-10: have you felt depressed or sad in the past week (yes, no)
ULSAM	71.0 (0.6)	7, 8-10 or ≥12 years in education	71.0 (0.6)	ICD-10: yes, no
WHII	55.5 (5.9)	continuous variable measuring years of education	60.9 (6.0)	GHQ-30: <4, ≥4

*Folk school is a Scandinavian school model where learning-by-doing is the basic educational philosophy of the schools

CIS-R: Clinical Interview Schedule-Revised; EWL: Eigenschaftswörterliste. This is a validated multidimensional tool consisting of a list of given adjectives describing the emotional status of a test person at the time of the interview (299). CES-D scale: Centre for Epidemiologic Studies Depression Scale. This is a short self-report scale design to measure depressive symptomatology based on the past week in the general population with higher summary scores indicating greater symptoms (300). A cut-off score of 3 has been shown to be clinically significant for 8-item questionnaire and cut-off of 16 for longer version (301); GDS: Geriatric Depression Scale. This is specifically designed for rating depression in the elderly. Cut-off score of 5 was used (302); HSCL-10: Hopkins symptom checklist-10 which is a self-reported symptom inventory consisting of symptoms commonly observed in the population (303); ICD-10: International classification of disease (304); GHQ: General Health Questionnaire (305)

Table 4.8: Comparison of 1958BC with additional cohorts

	1958BC	ASPS	ELSA	ESTHER	HBCS	PIVUS	Tromsø	ULSAM	WHII
Gender, %(N)									
Male	48.6 (3,086)	43.3 (358)	45.6 (2,525)	44.8 (4,369)	44.8 (811)	49.7 (502)	55.2 (6,804)	100 (1,194)	73.9 (3,807)
Female	51.4 (3,260)	56.7 (468)	54.4 (3,007)	55.2 (5,380)	55.2 (1,000)	50.3 (508)	44.8 (5,532)	0 (0)	26.1 (1,343)
Age at time of cognitive testing, mean (min-max)									
	50 (50-50)	65.6 (46.8-85.7)	66.1 (52.0-≥99)	74.0 (70-81)	68.1 (61.2-76.8)	70.2 (69.8-73.7)	64.5 (31-88)	71 (69.4-74.1)	60.9 (50.5-73.9)
25-hydroxyvitamin D, nmol/l, geometric mean (min-max)									
	52.9 (9.5-187.3)	-	-	46.8 (7.0-225.6)	59.3 (19.0-292.0)	54.3 (10.9-143.0)	55.5 (10.1-192.2)	65.8 (5.0-153.3)	-
missing, %(N)	7.2 (456)	-	-	2.5 (247)	42.9 (776)	1.0	42.0 (5,175)	0 (0)	-
Month of collection for 25(OH)D , %(N)									
January	8.1 (511)	-	-	12.2 (1,187)	7.8 (142)	8.4 (85)	3.2 (393)	1.0 (119)	-
February	7.1 (451)	-	-	9.6 (937)	3.8 (69)	8.9 (90)	4.5 (550)	9.2 (110)	-
March	6.3 (400)	-	-	7.2 (704)	7.2 (131)	12.2 (123)	4.4 (539)	7.0 (83)	-
April	5.2 (331)	-	-	6.7 (650)	7.0 (126)	9.6 (97)	2.7 (329)	8.0 (95)	-
May	5.9 (376)	-	-	6.9 (673)	6.6 (119)	10.5 (106)	3.7 (453)	6.4 (76)	-
June	8.9 (562)	-	-	7.3 (708)	3.2 (57)	8.5 (86)	3.4 (421)	4.8 (57)	-
July	7.5 (476)	-	-	6.6 (644)	0 (0)	0 (0)	0 (0)	0 (0)	-
August	6.6 (420)	-	-	10.1 (984)	4.0 (72)	6.3 (64)	3.3 (409)	4.5 (54)	-
September	12.9 (816)	-	-	9.6 (936)	4.7 (85)	8.8 (89)	3.8 (470)	15.2 (181)	-
October	13.6 (860)	-	-	7.4 (723)	4.6 (84)	10.0 (101)	3.6 (446)	14.7 (175)	-
November	13.2 (836)	-	-	10.2 (997)	5.1 (92)	9.4 (95)	2.5 (306)	13.5 (161)	-
December	3.9 (250)	-	-	6.1 (590)	3.3 (59)	7.3 (74)	2.0 (251)	7.0 (83)	-
missing	0.9 (57)	-	-	0.2 (16)	42.8 (775)	0 (0)	63.0 (7,769)	0 (0)	-
Depressive symptoms , %(N)									
No	92.2 (5,848)	1.7 (0-15)*	79.0 (4,372)	11.8 (1,148)	70.2 (1,271)	-	42.0 (5,178)	99.8 (1,192)	87.5 (4,505)
Yes	7.5 (477)		21.0 (1,160)	68.0 (6,627)†	14.8 (268)	-	1.3 (160)	0.2 (2)	11.2 (575)
missing	0.3 (21)	4.0 (33)	0 (0)	20.3 (1,974)	15.0 (272)	-	56.7 (6,998)	0 (0)	1.4 (70)

	1958BC	ASPS	ELSA	ESTHER	HBCS	PIVUS	Tromsø	ULSAM	WHII
Education‡ , %(N)									
Low to high	6.1 (387)	27.1 (224)	-	73.2 (7,132)	32.4 (586)	56.2 (568)	10.0 (0-48)	64.5 (770)	15.1 (0-35)
	12.3 (779)	39.8 (329)		13.7 (1,336)	19.3 (350)	17.8 (180)		17.5 (209)	
	27.0 (1,711)	23.1 (191)		10.7 (1,044)	26.3 (477)	24.9 (251)		18.0 (215)	
	16.1 (1,023)	9.4 (78)			21.8 (394)				
	26.9 (1,707)								
missing, %(N)	11.7 (739)	0.5 (4)		2.4 (237)	0.2 (4)	1.1 (11)	51.3 (6,323)	0 (0)	7.3 (376)

*ASPS depression N=793 mean (min-max).
†In ESTHER, depressive symptoms was coded as no=normal (*n*=1,148), yes=mild depressive (*n*=6,474) or severe depressive (*n*=153)
‡1958BC categories: none, <O-level, O-level, A-level or Higher, %(N);
ASPS categories: 9, 10 or 13 years in education, %(N);
ESTHER categories: ≤9, 10-11 or ≥12 years in education, %(N);
HBCS categories: Folk school/ elementary/ middle school, learning profession, elementary school or similar or lower/ higher university degree, %(N);
PIVUS categories: Primary school <9 years, Secondary school 9-12 years or university level >12 years, %(N);
Tromsø categories: years of education N=6,013, mean(min-max);
ULSAM categories: ≤7, 8-10 or ≥12 years in education, %(N);
WHII categories: Total number of years in education N=4,774 mean (min-max)

4.5 Summary

- Participants are primarily from the 1958 British birth cohort, with additional data from ASPS, ELSA, ESTHER, HBCS, PIVUS, Tromsø, ULSAM and WHII
- Serum 25-hydroxyvitamin D was the main exposure variable used
- Common mental disorders and cognitive function were the outcome variables used
- Genetic data regarding *APOE* genotypes and vitamin D-related SNPs were obtained and quality control measures were applied
- These data sources will support the aims of this thesis through the provision of well-defined cohorts and reliable and valid measurements of 25(OH)D concentrations, CMDs and cognitive function. The rich, detailed data obtained from these participants will facilitate both observational and genetic analyses so that dependable estimates and valid conclusions can be reached

Chapter 5 Observational study: Association between vitamin D and common mental disorder

5.1 Introduction

Chapter 1 highlighted the public health importance of reducing the burden of common mental disorders (CMD) in the population and the potential role and biological plausibility of vitamin D in this pursuit. CMDs can affect individuals at many time points throughout the life-course. However, one US study suggested that the median age of onset of a Diagnostic Statistical Manual-IV (DSM-IV) mood disorder is 30 years and a prevalence study in the UK indicated that those aged 45-54 years were the most likely to have significant neurotic symptoms (9, 61). Therefore, the current study focuses on the association between 25-hydroxyvitmain D (25(OH)D) and CMDs in mid-adulthood.

The relationship between 25(OH)D and CMDs in adulthood has been investigated by many observational epidemiological studies. In order to assess this epidemiological evidence, methodologies of a systematic review were applied, capturing data to July 22, 2013. Studies relating to 25(OH)D and CMDs were identified by searching in PubMed (*n*=1,473). Selected studies were limited to those published in the English language (*n*=1,227) and in human populations (*n*=977). Search terms used are given in **Appendix 3.1**.

There were 74 relevant studies identified following examination of the titles. From the 74, 37 were excluded following inspection of the abstracts (*n*=24 were reviews or commentary, *n*=6 used child or adolescent samples, *n*=7 were deemed irrelevant). Reference lists of previous systematic reviews were examined for additional relevant citations (178, 306-308) (*n*=2), resulting in a total of 39 studies. No full text was available for two of these studies; therefore the total number identified was 37. Study details are given in **Table 5.1**. Further information, including study setting, groups and adjustment for confounders can be found in **Appendix 3.2**.

Table 5.1: Systematic review: Vitamin D and CMDs

Author (year), country	Total N	Age (y)	Female (%)	Vitamin D, 25(OH)D, unless stated		Mental Health test	Results
				Method	Cut-point, nmol/l		
Cross-sectional							
Kwasky (2012)(309), US	139	(18-24)	100	MS		BDI ≥20	Correlation between 25(OH)D and BDI Total sample: r=0.005, *p*=0.95 African American: r=0.09, *p*=0.39 Caucasian: r=-0.15, *p*=0.26
Menkes (2012) (310), New Zealand	102	(18-65)	44.1	ECL	<50 <25	DSM-IV	Depression (n=17) Mean 47.5 (25.7), *p*=0.70, t-test to estimate the extent to which vitamin D levels in the study differed from the deficiency threshold of <50nmol/l
Zhao (2010) (311), US	3,916	≥20	51.2	RIA DiaSorin	Q1:<15ng/mL Q2: 15-20 Q3: 20-26 Q4: ≥26	PHQ-9 ≥10	OR with Q1 as reference Q2 (n=884) 1.24 (0.74, 2.10); Q3 0.92 (0.45, 1.88); Q4: 0.89 (0.45, 1.79), p_{trend}=0.62
Nanri (2009)(312), Japan	527	21-67	A 37.1 B 42.4	CBPA		CES-D ≥16	Workplace A (July)(n=159) OR with Q1 as reference Q2: .60 (0.20-1.76); Q3: 0.75 (0.27-2.08): Q4: (0.70 (0.24-2.05) p_{trend} =0.62 Workplace B (November)(n=368) OR with Q1 as reference Q2: 0.84 (0.45-1.58); Q3: 0.83 (0.44-1.58): Q4: (0.59 (0.30-1.15) p_{trend} =0.14
Armstrong (2007)(313)	75	(21-75)			<25 25-50 ≥50	HADS	<25nmol/l: 13.3%; 25-50nmol/l: 56.0%; ≥50nmol/l: 30.7 *Median (IQR) HADS* <25nmol/l: 31.0 (23.8-36.8) versus 25-50nmol/l 22.5 (17.0-26.2) versus ≥50nmol/l 23.5 (19.0-27.5) *p*<0.05
Jaddou (2012)(314), Jordan	4,002	≥25	74.8	RIA	Q1: >63.22 Q2: 42.31-	DASS21 ≥14	OR, with Q4 as referent, full adjustment Q1: 1.05 *p*=0.63; Q2 1.24, p0.03; Q3: 1.48,

Author (year), country	Total N	Age (y)	Female (%)	Vitamin D, 25(OH)D, unless stated		Mental Health test	Results
					63.22 Q3: 27.61-42.30 Q4: ≤27.60		*p*<0.001. p_{trend} =<0.001
Ganji (2010)(315), US	7,970	27 ±0.16 (15-39)			<50 50-75 >75	DIS	OR of current depressive episodes, with >75 as reference <500nmol/l: 1.85(0.90-3.81) 50-75nmol/l: 0.70(0.38-1.29), *p*=0.021
Knippenberg (2010)(316), Netherlands	59	44.2 ± 9.2	73	Unknown		HADS	*Pearson correlation*: r=-0.326, p=0.006, Pearson correlation *coefficient*: -0.247, p=0.078,
Kjærgaard (2011)(317), Norway	10,086	Smokers Female: 52.1 ± 11.4 Male: 55.4 ± 11.7 Non-smokers Female: 57.3 ± 13.0 Male: 57.5 ± 12.2	50.8	ECL	Smokers Q1: 21.7-71.4 Q2: 44.3-83.3 Q3: 54.9-96.8 Q4: 67.7-201.2 Non-smokers Q1: 6.7-54.5 Q2: 37.1-65.7 Q3: 45.9-77.9 Q4: 56.7-182.5	SCL-10 ≥1.85	Smokers (n=1,966) OR, Q1 as reference Q2: 0.98 (0.67-1.43): Q3: 0.67 (0.44-1.00); Q4: 0.59 (0.39-0.89), p_{trend} =0.003 Non-smokers (n=8,120) OR Q2: 0.88 (0.70-1.11): Q3: 0.77 (0.61-0.98); Q4: 0.74 (0.58-0.95), p_{trend} =0.01
Hoang (2011)(318), US	12,594	51.7±11	31.8	In-house		CES-D ≥10	OR for each 10ng/mL increase Total sample: 0.92 (0.87-0.97), *p*=0.002 with prior history: OR 0.90 (0.82-0.98), *p*=0.02 with no prior history: OR 0.95 (0.89-1.02), *p*=0.17
Pan (2009)(319), China	3262	(50-70)	-	RIA DiaSorin	Q1: 26.1 ± 5.9 Q2: 41.1 ± 4.1 Q3: 65.1 ± 6.0	CES-D ≥16	Prevalence of depressive symptoms Q1 (n=1087): 11.1% ; Q2 (n=1088): 10.4%; Q3 (n=1087) 7.2% OR with Q1 as reference Q2 : 1.38 (1.00-1.90); Q3 1.35 (0.94-1.96), p_{trend} =0.075

Author (year), country	Total N	Age (y)	Female (%)	Vitamin D, 25(OH)D, unless stated		Mental Health test	Results
Motsinger (2012)(320), US	15,954	79.2±4; 79.1±4; 79.1±4	100	FFQ	<400 IU/day 400-799 >800 IU/day	Mental health-related QOL	Least squares means and standard errors, fully adjusted <400IU/day: 46.4 (0.2); 400-799IU/day 46.8(0.2); 800IU/day 46.9 (0.3), *p*=0.002
Lee (2010)(321), Italy, Belgium, Poland, Sweden, UK, Spain, Hungary, Estonia	3,369	60 ±11	0	RIA	≥75 50-74.9 25-49.9 <25	BDI-II >14	Mean 25(OH)D (SD) No depression (n=2719) 63.6 (31.6) versus depressed (n=432): 52.3 (25.4), *p*<0.001 Percentage change in BDI score (95% CI) per 10nmol/l decrease 3.2 (1.1 to 5.5), *p*<0.05 Percentage change in BDI score (95% CI) with ≥75nmol/l category as reference 50-74.9nmol/l: 10.2(-1.2, 22.9); 25-49.9 nmol/l: 22.1 (3.7 to 43.7): <25nmol/l: 42.3 (20.5, 68.1) p_{trend} =0.01
Stewart (2010)(322), UK	2,070	≥65	54.1	RIA DiaSorin	<10ng/ML	GDS ≥3	OR (95% CI), for fully adjusted 1.46 (1.02 to 2.08), *p*=0.04
Hoogendijk (2008)(323), Netherlands	1282	(65-95)		CBPA	Q1:≤14.7 ng/ml Q2: 14.7-20.4 Q3: 20.4-27.4 Q4: >27.4	CES-D, ≥16 DIS	CES-D Scale score: β(95% CI) per ng/mL 8.0 (15.2 to 0.8), *p*=0.03 Compared to Q1: Q2: -0.01(-0.12 to 1.11), *p*=0.99 Q3: -1.09 (-2.25 to 0.07), *p*=0.07 Q4: -1.07(-2.28 to 0.14)
Wilkins (2006)(324), US	80	74.8±7.7	62.5	Ria DiaSorin	<25 25-50 ≥50	Clinician - DSM-IC	Mean depressive symptoms score (SD): <25 (n=13): 2.77 (2.62); 25-50 (n=33) 2.26 (2.29); ≥50 (n=34): 2.24 (2.14) OR for Mood disorder with≥50 as reference: <25: 11.69 (2.04 to 66.86); 25-50: 2.56 (0.63 to10.51), p_{trend}=0.21

Author (year), country	Total N	Age (y)	Female (%)	Vitamin D, 25(OH)D, unless stated	Mental Health test	Results
Johnson (2008)(325), US	158	77	81	Ria DiaSorin <25 25-50 ≥50	GDS, ≥11	Geriatric Depression Scale ≥11 (n=98) <25nmol/l: 56%; 25-<50nmol/l 12%; ≥50nmol/l 23% <25 versus ≥25, *p*= 0.02 <50 versus ≥50, *p*= 0.45
Case-control						
Cizza (2012)(326), Italy	136	(21-45)	Case: 100 Control:100	Unknown	MDD DSM-IV	25(OH)D mean (SD) ng/mL: Controls: 34.20(2.27) MSS: 27.57(1.11) *p*<0.01
Eskandari (2007)(327)	133	Case: 35 ±6.9 Control: 35±6.8	Case: 100 Control: 100	CIA	DSM-IV	25(OH)D mean (sd) ng/mL Control (n=41): 34 (14) Cases (n=75): 27 (10.1), *p*=0.002
Schneider (2000)(328), Germany	120	Cases: 38.9±2.1 48.7±2.2 57.6±3.5 Control: 38.8±3.2	Cases: 44 16 56 Control: 39	25(OH)D $1,25(OH)_2$ D	ICD-10 DSM-III-R	25(OH)D, mean ± SD (pg/ml) Healthy controls (n=31): 45.9 ± 19.8 Depression (n=25): 37.3 ± 26.1 Schizophrenia (n=34): 35.1 ± 26.1 Alcoholism (n=30): 45.6 ± 35.3 1,25-dihydroxyvitamin D, mean ± SD (ng/ml) Healthy controls (n=31): 39.4 ± 15.7 Depression (n=25): 29.2 ± 9.1 Schizophrenia (n=34): 29.0 ± 11.6 Alcoholism (n=30): 38.3 ± 16.9
Oren (1994)(329), US	15	Case: 40±7 Control: 40±7	Case: 60 Control: 60	Calcitriol CPBA	DSM-III-R	1,25-dihydroxyvitamin D pg/ml, mean (SD) Patients: in the dark: 35 (14); in the light: 40 (16) Controls: in the dark: 39 (14). In the light 43(17), *p*>0.05
Michelson (1996) (330), US	24	Case: 41±8 Control: 41±7	Case: 100 Control: 100	Calcitriol (column chromatography 25(OH)D	DSM-III	1,25-dihydroxyvitamin D pg/ml, mean (SD) Cases: 50 (20) Controls: 44(19), *p*=0.25 25(OH)D ng/ml, mean (SD) Cases: 39 (18)

Author (year), country	Total N	Age (y)	Female (%)	Vitamin D, 25(OH)D, unless stated		Mental Health test	Results
				CPBA			Controls: 35(16), *p*=0.44
Berk (2008)(331), Australia	53	Case:44.0 (36.0-52.0) Control:48.0 (33.6-71.4)	Case: 100 Control: 100	RIA DiaSorin	≤50 ≤25	Psychiatric inpatients	Age and season adjusted geometric mean Cases: 46.4 (38.6-54.9) Control: 65.3 (63.2-67.4), *p*<0.001
Herrán (2000)(332), Spain	38	Case: 44.7 ±12.1 Control: 43.5 ±8.4	Case: 100 Control: 100	RIA DiaSorin		ICD-10	Mean (SD), ng/ml Patients: 27.3 (16.1) Controls: 23.0 (7.8), *p*=0.20
Jorde (2006)(333), Norway	84 (148 total cohort)	Case: 62.3±15.3; Control: 63.5 ±13.2	Case: 42.8 Control: 38.0	RIA DiaSorin		BDI	Mean ± SD score for BDI (1-13) SHPT (n=21): 2.67 ±3.04 Controls (n=63): 2.03±2.28, *p*<0.05 Mean ± SD) score for BDI (14-21) SHPT (n=21): 2.38 ±1.56 Controls (n=63): 2.42±2.67, *p*>0.05 Mean ± SD score for BDI (total) SHPT (n=21): 5.05 ±3.29 Controls (n=63): 4.45±4.46, *p*>0.05
Cohort							
Bertone-Johnson (2011)(334), US	81,189	(50-79)	100	FFQ	≥800IU/day ≥400IU/day <100IU/day	Burnam scale, ≥0.06	Prevalence OR (95% CI), <100IU/day as reference ≥800 IU/day: 0.79 (0.71 to 0.89), p_{trend}<0.001 Prospective OR (95% CI), <100IU/day as reference ≥800 IU/day: 0.80 (0.6 to 0.95), p_{trend} =0.001
Chan (2011)(335), China	939	>65	0	RIA DiaSorin	Q1: ≤ Q2: 64-76 Q3: 77-91 Q4: ≥92	GDS ≥8	Baseline with Q1 as reference Q2:1.39 (0.76-2.54) Q3: 0.48 (0.23-1.01) Q4:0.46 (0.22-0.98), p_{trend} =0.004 Follow-up Q2:5.15 (1.17-22.70) Q3: 1.98 (0.38-10.35) Q4:1.64 (0.30 -9.03), p_{trend} =0.82
Milaneschi (2010)(336),	954	≥65	55.7	RIA	<50	CES-D ≥16	Increase in CES-D score: 3-year follow-up

Author (year), country	Total N	Age (y)	Female (%)	Vitamin D, 25(OH)D, unless stated		Mental Health test	Results
Italy				DiaSorin			<u><50nmol/l versus ≥50nmol/l</u> Women: 2.1 *p*=0.002 Men 1.9 *p*=0.01 <u>Increase in CES-D score: 6-year follow-up</u> <u><50nmol/l versus ≥50nmol/l</u> Women 2.2, *p*=0.04 Men: 1.1, *p*=0.20 <u>HR (95% CI) for <50nmol/l versus ≥50nmol/l</u> Women: 2.0 (1.2 to 3.2), *p*=0.005 Men: 1.6 (0.9- to.8), *p*=0.1
May (2010)(337), US	7,358	73.1±10.2	58.8	CIA	>50ng/mL 31-50 16-30 ≤15	ICD-9	<u>HR (95% CI) with >50ng/mL l as reference, fully adjusted</u> ≤15ng/mL: (n=1325): 2.70 (1.35 to 5.40) 16-30ng/mL: 2.15 (1.10 to 4.21) 31-50ng/mL: 1.95 (0.99 to3.87) , p_{trend} =0.06
Randomised-controlled trials							
Lansdowne (1998)(338), Australia	44	(18-43)	77.2		Treatment 1 n=150; 400IU/day and 9000IU vitamin A/day Treatment 2 n=142; 8000IU/day Control: n=149 0IU/day and 10,000IU vitamin A/day	PANAS	*Difference in PA scale between groups versus control*: *p*<0.001 *Difference in NA scale between groups versus control*: p<0.05

Author (year), country	Total N	Age (y)	Female (%)	Vitamin D, 25(OH)D, unless stated		Mental Health test	Results
Dean (2011)(339), Australia	128	Treatment 24.45 ± 2.96 Control: 22.06± 2.74	Treatment 61.9 Control: 52.3	MS	Treatment n=63 5000IU/day Control: n=65 placebo/day	BDI STAI	Baseline *BDI score (SD)*: Treatment: 7.24 (7.82); Placebo 5.72 (5.56), *p*>0.05 *STAI score (SD)*: Treatment: 36.29 (10.10): Placebo 34.15 (8.31), *p*>0.05 6-week Follow-up *BDI score (SD)*: Treatment: 6.40 (0.85); Placebo 5.38 (0.83), *p*=0.51 *STAI score (SD)*: Treatment: 36.68 (1.21): Placebo 36.08 (1.19), *p*=0.23
Harris (1993)(340), US	250	(43-72)	100			POMS	No correlation between 1,25-dihydroxyvitamin D or 25(OH)D with POMS scores. No differences in POMS scores of those with a supplement versus placebo
Jorde (2008)(341), Norway	441	Treatment 1 46 (21-70) Treatment 2 48.5 (23-70 Control: 48 (24-69)	Treatment 1 62 Treatment 2 64 Control: 65.8		Treatment 1 n=150; 20,000 and 20,000IU/week Treatment 2 n=142; 20,000IU and placebo/week Control: n=149 2 placebo/week	BDI 1-13 14-21	Baseline BDI total score Treatment 1: 4.5 Treatment 2: 5.0 Control: 4.0, no significant difference between three treatment groups 12 Month BDI total score Treatment 1: 3.0, *p*<0.01 compared to baseline Treatment 2: 4.0, *p*<0.01 compared to baseline Control:3.8 *p*>0.05 compared to baseline
Bertone-Johnson (2011)(334), US	36282	(50-79)	100	FFQ	Treatment n=18,176 400IU/day with 1,000mg calcium Control: n=12,421	Burnam scale, ≥0.06	Burnam depression scale score Treatment: *<0.06*: n=16,401 (90.2*%); ≥0.06*: n=1,708 (9.4%) Control: *<0.06* n=16,244(89.7%);≥*0.0 6* n=1,790 (9.9%), *p*=0.25

Author (year), country	Total N	Age (y)	Female (%)	Vitamin D, 25(OH)D, unless stated		Mental Health test	Results
					placebo/day		Mean change in Buranm Scale between year 1 and 3 Treatment (n=1,109):0.007 Control (n=1,143) 0 Multivariate OR for Burnam score ≥0.06 Treatment (n=119 cases, n=997 non-cases): Control (n=108 cases, 1,039 non-cases) 0; 1.16 (0.86, 1.56)
Kjærgaard (2012)(342), Norway	344	Treatment 52.6 (10.3) Control 52.1 (9.2)	Treatment 51.2 Control 52.0	LC-MS	Treatment n=180 with 25(OH)D <55nmol/l; 2 x20 000IU/week Control: n=75 with 25(OH)D >70nmol/l; 2 placebo 000IU/week	BDI HADS SPAQ MADRS SCID-CV	Serum 25(OH)D at baseline Case: 40.1 (8.8) versus Control: 92.3 (15.8), *p*<0.05, BDI median at baseline Case: 4 (0-49) versus Control: 3 (0-23), *p*>0.05 HADS median at baseline Case: 4 (0-25) versus Control: 3 (0-16), *p*<0.05 MADRS median at baseline Case: 2 (0-26) versus Control: 1(0-13), *p*<0.05 GSS, median at baseline Case: 6 (0-21) versus Control: 5 (0-15), *p*>0.05 Intervention: BDI median Treatment (n=120): Baseline 4 (0-31); 6-months 3(0-35), *p*=0.92 Placebo (n=110): Baseline 4 (0-49); 6-months 2 (0-35) Intervention: HADS median Treatment: Baseline 5 (0-22); 6-months 4 (0-20), *p*=0.21 Placebo: Baseline 4 (0-25); 6-months 3 (0-31) Intervention: MADRS median Treatment: Baseline 2 (0-26); 6-months 2 (0-15), *p*=0.34 Placebo: Baseline 2 (0-25); 6-months 1 (0-28)

Author (year), country	Total N	Age (y)	Female (%)	Vitamin D, 25(OH)D, unless stated	Mental Health test	Results
						Intervention: GSS median Treatment: Baseline 6 (0-16); 6-months 4 (0-14), *p*=0.73 Placebo: Baseline 6 (0-21); 6-months 6 (0-19)
Sanders (2011)(343), Australia	2034	≥70	100	Treatment n=1018 500,000IU every autumn/winter Control: n=1016 placebo	GHQ (≥3) SF-12 WHO Well-being Index PGI-I	Estimated treatment effects *GHQ (OR, (95% CI)):* 1.06 (0.81 to 1.37), *p*=0.69 SF-12 (β*95% CI)*): -0.14(-1.00 to 0.72) p=0.75 *WHO Well-being score (OR (95% CI)*) -0.04 (-0.52 to 1.44), *p*=0.96 WHO *poor well-being score (OR* (*95% CI)*): 0.85 ((0.45 to 1.61), *p*=0.63
Before-after study						
Shipowick (2009)(344), US	9	42.2 ±13.17	100		BDI-II 0-13 normal 14-19 mild depression 20-28 moderate depression 29-63 severe depression	Mean (Sd) vitamin D ng/ml Baseline: 21.8 (8.33) Follow-up: 48.2 (20.1), *p*=0.009 Mean BDI-II (SD) Baseline: 37.8 (4.79) Follow-up: 21.2 (11.07), *p*=0.02

BDI (Beck Depression Inventory); BMI (Body Mass Index); CBPA (competitive protein-binding assay); CCLS (Cooper Centre Longitudinal Study); CES-D (Centre for Epidemiologic Studies Depression Scale); CIA (Chemiluminescent ImmunoAssay); CVD (Cardiovascular disease); DASS21 (Depression Anxiety Stress Scales); DIS (Diagnostic Statistical Manual); EMAS (European Male Ageing Study); FFQ (Food Frequency Questionnaire); GDS (Geriatric Depression Scale); CHQ (General Health Questionnaire); GOS (Geelong Osteoporosis Study); GSS (Global Seasonality Score); HADS (Hospital Anxiety and Depression Scale); HR (Hazard Ratio); HSE (Health Survey for England); ICD (International Classification of Disease); IWHS (Iowa Women's' Health Study); MADRS (The Montogmery--Åsberg Depression Rating Scale); MDD (Major Depressive disorder); MS (Mass spectrometry); MS (Multiple Sclerosis); NHANES (National Health and Nutrition Examination Survey); NHAPC (Nutrition and Health of Ageing Population in China); OAANP (Older Americans Act Nutrition Program); OR (Odds Ratio); PANAS (Positive and Negative Affect Schedule); PGI-I (Patient Global Impression Improvement)' PHQ-9 (Patient Health Questionnaire-9 diagnostic algorithm); POMS (Profile of Mood States); POWER (Premenopausal, Osteoporosis, Women, Alendronate, Depression); QOL (Quality of life); SAD (Seasonal affective disorder); SCID-CV (Structured Clinical Interview for DSM-IV Axis I disorders-clinical Version); SCL-10 (Hopkins symptoms check list 10); SF-12 (12-item Short From Health Survey); SHPT (Secondary Hyperparathyroidism); SPAQ(Seasonal Pattern Assessment Scale); STAI (The State-Trait Anxiety Inventory); WHI (Women's Health Initiative); WHO (world Health Organisation)

The majority of studies conducted to date were cross-sectional in design (*n*=17). Of these, 64.7% reported a significant association between lower 25(OH)D concentrations or intake and prevalent CMDs (mainly depressive symptoms) (313-315, 317, 318, 320-325). For instance, one Norwegian study conducted in 8,120 middle-aged non-smokers found that the odds for having depression or significant mental stress in those with the highest levels of 25(OH)D (56.7-182nmol/l) were 26% lower (95% CI 0.1% to 42%) than those with the lowest 25(OH)D concentrations (6.7-54.5nmol/l). In the same study, smokers (*n*=1,966) in the highest quartile of 25(OH)D also had 41% lower odds (95% CI 11% to 61%) of depression than those in the lowest quartile of 25(OH)D. These results were adjusted for age, gender, BMI, physical activity, alcohol intake, education, marital status, kidney function and chronic disease (317).

The review identified eight case-control studies, of which five reported significantly lower 25(OH)D concentrations in those with depressive disorders compared with controls (326-328, 331, 333).

Three of the four prospective studies showed a significant association between either low vitamin D intake or low 25(OH)D concentrations and subsequent depressive symptoms (334, 336, 337), while the fourth study reported a cross-sectional but not prospective association (335). Follow-up time for these studies ranged from 1 to 6 years.

Of the seven randomised controlled trials identified, two reported a significant improvement in depressive symptoms with vitamin D supplementation (338, 341). For example, in a Norwegian study of participants with a BMI of 28-47 kg/m^2, supplementation with high doses of vitamin D (i.e. either 20,000IU or 40,000IU vitamin D per week) was found to improve scores on the Beck Depression Inventory (BDI) over the course of 1 year (341). An additional study carried out amongst women in the US found that supplementation with 5,000IU vitamin D over the course of 8 weeks was associated with an average decline of 10 points on the BDI (344). Conversely, in an Australian study of 2,034 older females (aged ≥70 years), those supplemented with 500,000IU (*n*=1018) every autumn/winter did not show an improvement in CMDs symptoms as measured

on the General Health Questionnaire, 12-Item Short Form Health Survey, World Health Organisation well-being index and the Patient Global Impression Improvement over a period of three to five years (343).

Findings from observational epidemiologic studies remain equivocal. These heterogeneous results may be partly attributed to study design issues. Study populations differed for example, some studies used only female populations (309, 320) where others focused exclusively on males (321, 335). Furthermore, some studies focused on specific populations, for example, those with multiple sclerosis (316), with cardiovascular disease (337), or with fibromyalgia (313). Comparability between studies may also have been reduced as a result of the variety of diagnostic criteria and instruments used to measure depressive symptoms and the different assays used to assess 25(OH)D concentrations.

A meta-analysis was conducted in 2013 to evaluate the association between 25(OH)D and the risk of depressive symptoms (184). The authors of this paper used data from cross-sectional and cohort studies consisting of more than 50,000 study participants. Results indicated that a 10ng/ml increase in 25(OH)D (i.e. a 25nmol/l increase) was associated with a 4% decrease in the risk of depressive symptoms in cross-sectional studies and an 8% decrease in the incidence of depressive symptoms in cohort studies.

This promising inverse relationship between 25(OH)D and CMDs identified from observational studies requires further scrutiny before a conclusion can be reached. Many studies have focused on depression, while the relationships between 25(OH)D and other types of CMDs have been neglected. There are also varieties of social and lifestyle factors that may potentially confound the association between 25(OH)D and CMDs, some of which were controlled for in previous studies. However, there has been a lack of investigation to determine if lifestyle factors known to affect 25(OH)D concentrations, such as time spent outdoors, sun exposure habits or vitamin D supplements (26), differ between those with and without CMDs. Differences in behaviour of those with CMDs could explain the associations observed for low 25(OH)D concentrations. Therefore, this chapter uses an observational study to examine if an association

between 25(OH)D and CMDs remains despite the potential changes in vitamin D-related lifestyle factors between those with and without CMDs (**Chapter 2**).

5.2 Data and methods

All data used in this chapter, including how 25(OH)D concentrations were collected, how CMDs were assessed and details of covariates, were described in **Chapter 4**. Here, I will give a brief overview of the relevant data for this chapter.

5.2.1 Participants

Information was obtained from participants of the 1958 British birth cohort (1958BC), which is described in detail in **Chapter 4**. Data came primarily from the 45 year biomedical survey. Participants without measurements for vitamin D (*n*=711) and with no data on CMDs (*n*=30) at 45 years, as well as 1 pregnant woman and participants of non-European ancestry were excluded (*n*=159). Consequently, 7,401 participants were included for cross-sectional analysis. Participants were eligible for prospective analysis if they had information on vitamin D status and CMDs at 45 years and information on depressive symptoms at 50 years (*n*=5,966). **Figure 5.1** illustrates participant selection.

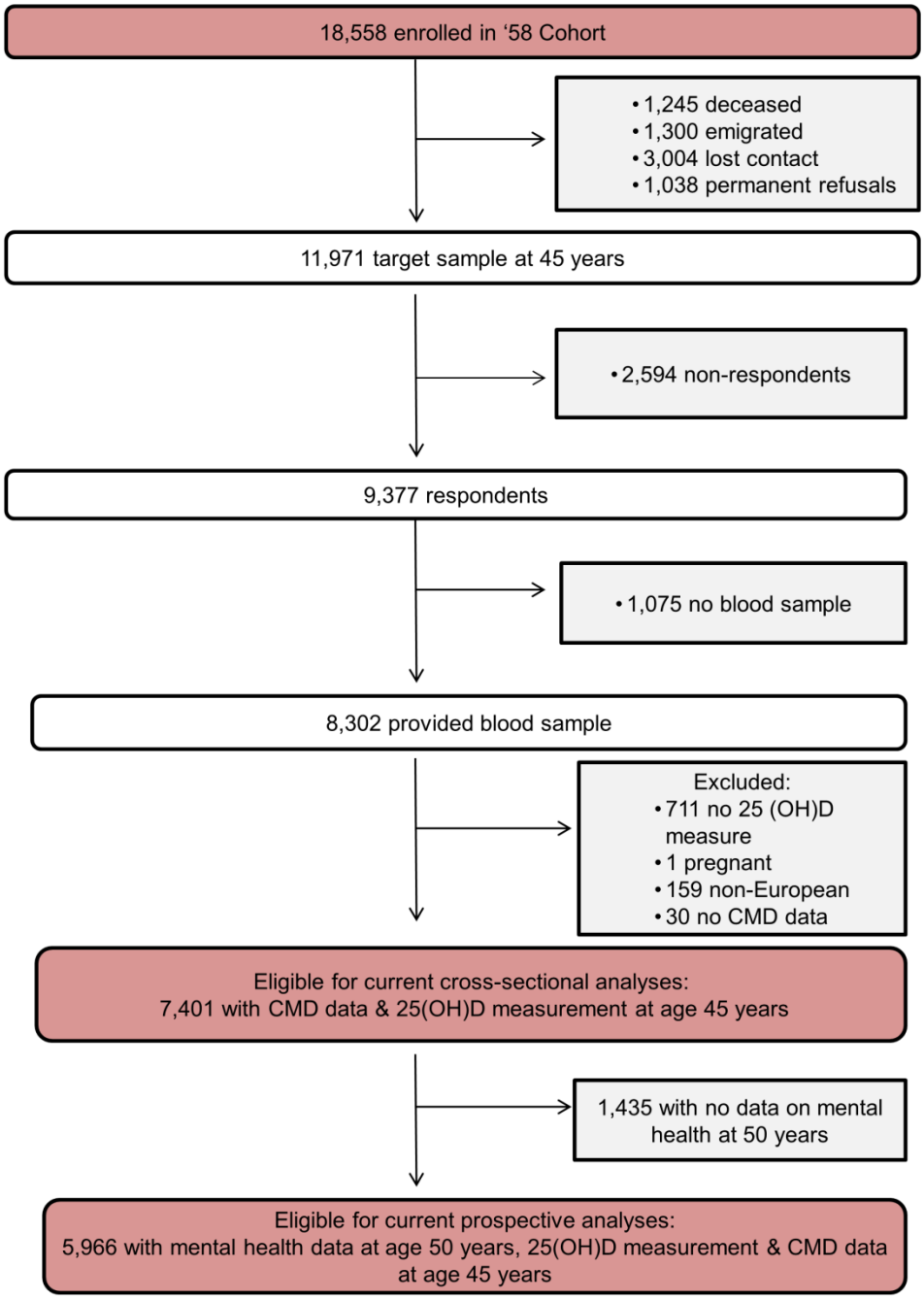

Figure 5.1: Participant selection for study on 25(OH)D and CMDs

5.2.2 Variables

25(OH)D concentrations were used to assess vitamin D status. Categorisation of 25(OH)D was based on previously established thresholds (**Box 5.1)** (42, 44).

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Box 5.1 25-Hydroxyvitamin D thresholds

- <25nmol/l
- ≥25 to <50nmol/l
- ≥50 to <75nmol/l
- ≥75 to <100nmol/l
- ≥100nmol/l

Symptoms of CMDs were assessed when participants were 45 and 50 years (**Chapter 4**). Briefly, at 45 years, CMDs were assessed using the Clinical Interview Schedule Revised (CIS-R) (240). The presence of depressive, anxiety, panic and phobia symptoms in the past week were assessed and individually summarised on a scale of zero to four. A score of ≥2 was used to indicate the presence of a clinically relevant CMD and <2 symptoms indicated no CMD (345).

At 50 years, the presence of depressive symptoms in the past four weeks was identified using the five question Mental Health Inventory (MHI-5) (244). Responses were summed and standardized to a 0-100 scale with lower scores indicating worse mental health (244). Although the MHI-5 was designed as a general mental health measure, here a cut off of ≤52 will be used to indicate the presence of depressive symptoms (247).

All other covariates used in these analyses are described in **Table 5.2**.

Table 5.2: Covariates from 1958BC for study on 25(OH)D and CMDs

Covariate	Age (y) of measurement	Description
Season of blood collection	45	Winter (December-February), spring (March-May), summer (June-August) or autumn (September-November)
Region of residence	46	Southern England and Channel Islands (South), Middle England and Wales (Middle), Northern England and Isle of Man (North) or Scotland.
SEP* in childhood	Father's occupation at birth (or at 7 years if missing)	Professional and managerial (I and II), non-manual (IIINM), manual (IIIM) and partly or unskilled (IV and V).
SEP in adulthood	42	I and II, IIINM, III, IV and V and unknown.
Smoking	42	Never, ex-smoker, 1-19 per day or ≥20 per day
Alcohol	45	Non-drinker, light drinker (<7 units per week), moderate (7-14 units per week), heavy (14-21 units per week) or very heavy (>21 units per week).
BMI (kg/m^2)	45	Participants weight (kg) divided by height (m^2). Obesity=BMI ≥30kg/m^2.
Physical activity	42	<2-3 times/month, 1 time per week, 2-3 times per week or 4-7 times per week
Frequency of consumption of oily fish (i.e. salmon, trout, mackerel, sardines or fresh tuna)	45	Weekly or less than weekly
Frequency of consumption of margarine	45	Weekly or less than weekly
Supplements of cod liver, fish oil or others containing vitamin D	45	Daily or less than daily
Amount of time spent outside during the past month	45	≥3 or <3 hours per day
Leisure time spent using the TV or PC	45	≥3 or <3 hours per day
Frequency of suncover usage	45	Most of the time or rarely
Blistering after sunburn	45	Often, rarely, sometimes or never
Seeking suntan	45	Often, rarely, sometimes or never

*SEP; socioeconomic position

5.2.3 Statistical analysis

Descriptive statistics

Descriptive statistics (**Chapter 3**) were used to establish the distribution of 25(OH)D concentrations and presence of CMDs in the eligible sample. The distribution of 25(OH)D was found to be slightly left skewed (**Appendix 3.3**), therefore, a natural logarithmic (ln) transformation was applied to improve approximation of a normal distribution and geometric means are presented. Comorbidity between types of CMDs at 45 years was examined using the chi-square test. The association between demographic characteristics according to the presence of a CMD was described and examined using gender-adjusted logistic regression models with CMDs as the binary outcome.

CMDs and vitamin D-related lifestyles

The relationships between types of CMDs and vitamin D-related lifestyle behaviours (i.e. time spent outside, time spent watching TV/using a PC, use of suncover, blistering after sunburn, actively seeking suntan, use of vitamin D-containing supplements, consumption of oily fish and consumption of margarine, **table 5.2**) were assessed using logistic regression models (**Chapter 3**) with vitamin D-related lifestyle behaviours as the binary outcome. These models were adjusted for gender and socioeconomic position (SEP) in adulthood.

Cross-sectional association between 25(OH)D and CMDs

In order to investigate the cross-sectional association between 25(OH)D and types of CMDs, multiple logistic regression models were used. Here 25(OH)D was used as a categorical exposure (i.e. <25, 25-49, 50-74, 75-99, ≥100nmol/l) whereby <25nmol/l 25(OH)D was used as the reference category and types of CMDs were the binary outcome.

Regression models were adjusted for:

1) Gender and season of blood collection
2) SEP at birth and adulthood in addition to adjustment 1

3) BMI in addition to adjustment 1 and 2
4) Lifestyle factors found to be associated with CMDs; smoking, physical activity, PC/TV leisure time, suncover, blistering after sunburn and actively seeking suntan in addition to adjustment 1-3

To examine the possibility of a more complex relationship between 25(OH)D and CMDs, the presence of a non-linear association was investigated (**Chapter 3**). The non-linear association was explored by including the curvature term of 25(OH)D i.e. ($25(OH)D^2$) in the model. The mean probability of having depressive symptoms at 45 years, over a range of 25(OH)D concentrations, was predicted from the multiple regression models found to be most appropriate for the data (i.e. linear or non-linear). These predicted probabilities were used to construct the graphs displayed in **Figure 5.5**.

Prospective association between 25(OH)D and depressive symptoms

The prospective association between 25(OH)D and depressive symptoms at 50 years was investigated using multiple logistic regression models with categorical 25(OH)D as the exposure and presence of depressive symptoms as the outcome. Models at 50 years were adjusted for the same covariates as in the cross-sectional analyses, and for the presence of any CMD at 45 years.

As with cross-sectional analyses, presence of non-linearity between 25(OH)D and depressive symptoms was examined by including the curvature term of 25(OH)D. The mean probability of having depressive symptoms at 50 years was predicted from the statistical model found to be most appropriate for the data (i.e. linear or non-linear). These predicted probabilities were used to construct the graphs displayed in **Figure 5.6.**

Tests for interaction between gender and 25(OH)D with symptoms of CMDs at 45 or 50 years (**Chapter 3**) were conducted. Since there was no evidence of interaction by gender in either cross-sectional or prospective analyses, combined, gender adjusted analyses are presented.

Additional analyses

Additional analyses were conducted to further investigate the relationship between 25(OH)D and symptoms of CMDs and increase confidence in the results (**Chapter 3**). In order to explore how reverse causality may affect the results of the cross-sectional association between 25(OH)D and types of CMDs, linear regression models with naturally log-transformed 25(OH)D as the outcome and CMDs as the exposure were applied. These models were adjusted for the same covariates as described for cross-sectional analyses.

Furthermore, sensitivity analysis was conducted to investigate the use of different thresholds (i.e. MHI-5 ≤60 and ≤75) for defining depressive symptoms at 50 years (**Chapter 3**).

81.1% (*n*=5,999) of participants had complete data for all covariates. Missing values ranged from 0.22% (*n*=16) for alcohol to 11.3% (*n*=836) for blistering after sunburn (**Figure 5.2**). Multiple imputation was used to correct for missing data on covariates and sample inverse probability weights were used to account for potential selection bias (**Chapter 3**). Imputed results are presented, with complete and weighted results in **Appendices 3.7** to **3.14**. A comparison of results from complete-case, weighted-case and imputed datasets is given in **Table 5.10**.

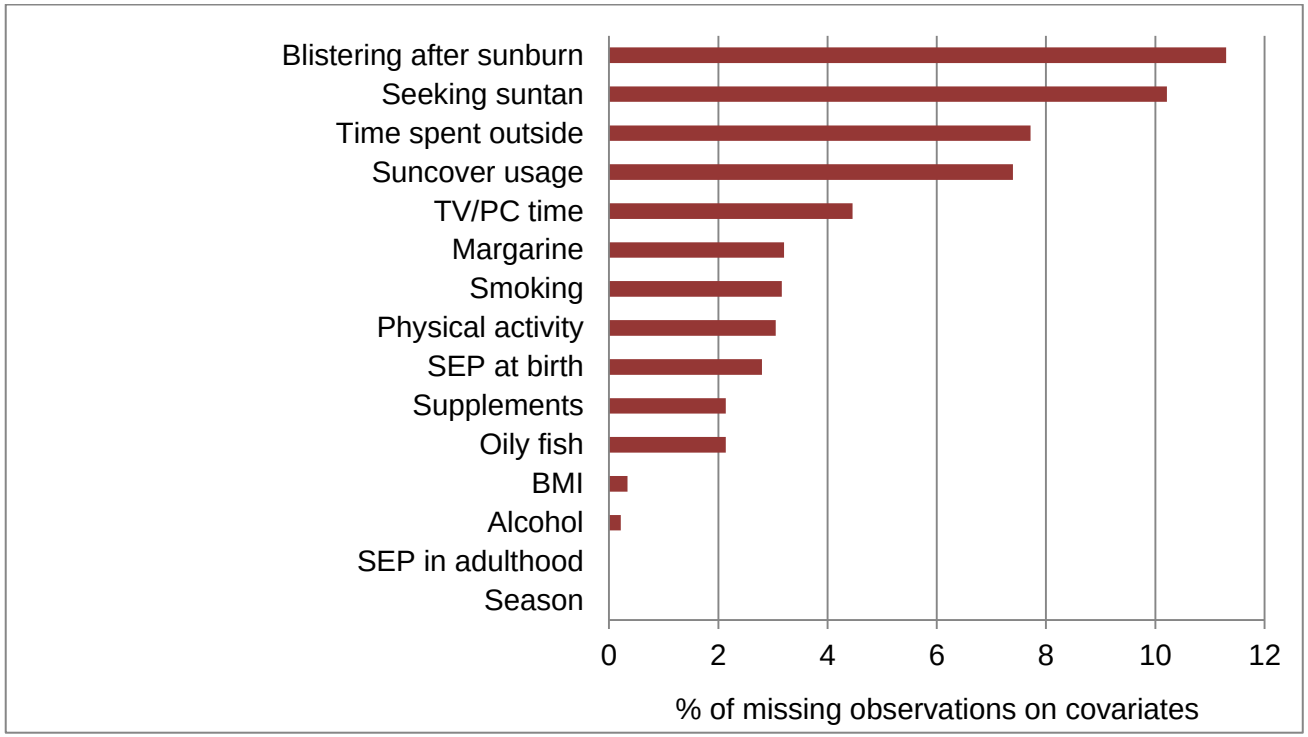


Figure 5.2: Proportion of missing covariate observations for study on 25(OH)D and CMDs (N=7,401)

5.3 Results

5.3.1 Descriptive characteristics

Women accounted for almost half of the study sample (49.9%). Serum 25(OH)D concentrations ranged from 9.5 to 230.7nmol/l. Geometric mean 25(OH)D concentrations were found to be slightly higher in men (53.1 nmol/l, 95% CI 52.3 to 53.9) than women (51.2 nmol/l, 95% CI 50.4 to 52.0nmol/l). As expected, average 25(OH)D concentrations were substantially higher in summer/autumn (60.3nmol/l, 95% CI 59.6 to 61.0nmol/l) compared with winter/spring (41.2nmol/l, 95% CI 40.5 to 41.9 nmol/l, $p<0.001$ adjusted for gender).

Symptoms of depression were the most prevalent CMD at 45 years (n=595, 8.0%), followed by anxiety (n=506, 6.8%), phobia (n=304, 4.1%) and panic symptoms (n=107, 1.5%). There was evidence of comorbidity ($p<0.001$ between all types of CMD) which is displayed in **Figure 5.3**.

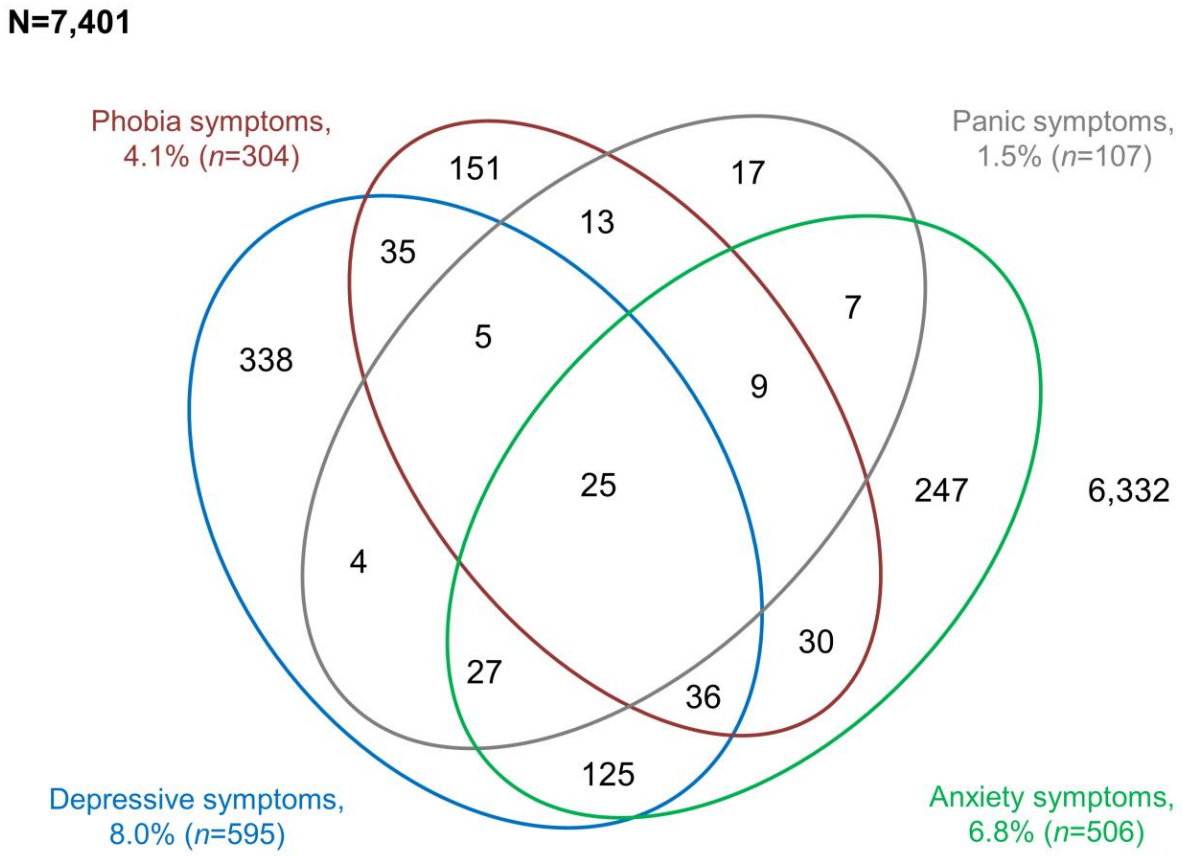


Figure 5.3: Comorbidity of symptoms of CMDs at 45 years

Of the participants that answered the MHI-5 at 50 years (*n*=5,966), 12.2% (*n*=725) had depressive symptoms (MHI-5 ≤52). Additionally, 32.6% (*n*=236) of participants with depressive symptoms at 50 years were classified as having symptoms of CMDs at 45 years. Women were significantly more likely to be affected by symptoms of CMDs at both 45 and 50 years than men ($p<0.001$). **Figure 5.4** illustrates the geometric mean of 25(OH)D by symptoms of CMDs at 45 years and presence of depressive symptoms at 50 years.

As seen in **Table 5.3**, participant characteristics varied according to symptoms of CMDs. Participants with symptoms of CMDs were less physically active and had poorer lifestyle habits compared with those without symptoms of CMDs. For example, individuals with depressive, panic and phobia symptoms were more likely to be obese ($p\leq0.04$ for all, adjusted for gender) compared with those without symptoms of CMDs. Participants with depressive and anxiety symptoms were overrepresented in groups who rarely exercised and who exercised the most. Participants with symptoms of CMDs were also more likely than others to smoke heavily ($p<0.001$ for all) and be in lower SEP ($p\leq0.002$ for all).

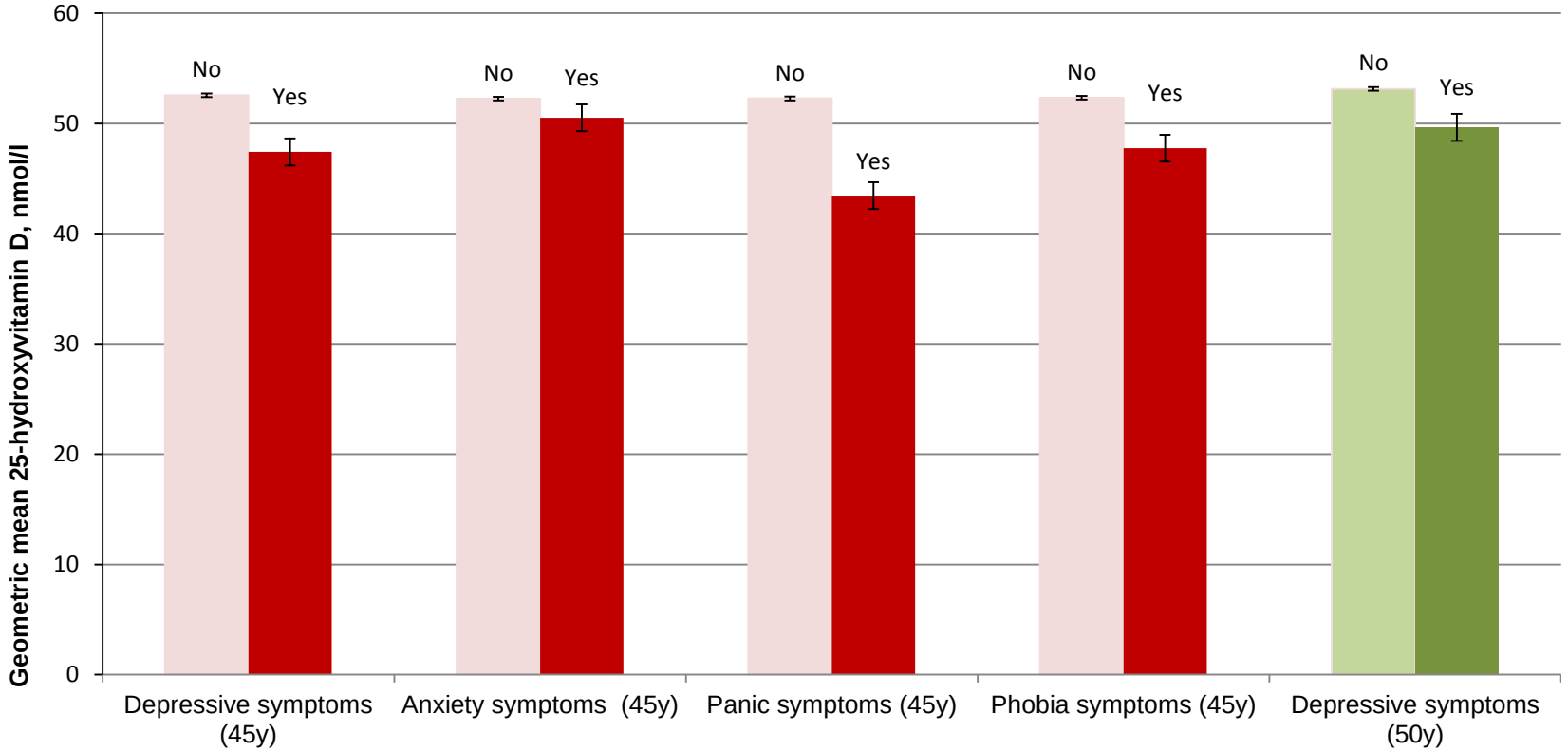


Figure 5.4: Geometric mean of 25(OH)D with standard error according to CMDs at 45 years and depressive symptoms at 50 years

Table 5.3: Characteristics of participants with ≥2 symptoms of CMDs at 45 years

	Total (n=7,401)	Depression (n=595) % (n)	Anxiety (n=506) % (n)	Panic (n=107) % (n)	Phobia (n=304) % (n)
Gender					
Male	3,705	7.0 (259)	5.4 (201)	0.8 (28)	3.1 (114)
Female	3,696	9.1 (336)	8.3 (305)	2.1 (79)	5.1 (190)
Combined	7,401	8.0 (595)	6.8 (506)	1.5 (107)	4.1 (304)
p		0.001	<0.001	<0.001	<0.001
Season					
Winter	1,286	8.4 (108)	6.6 (85)	1.9 (24)	3.3 (42)
Spring	1,546	7.8 (120)	5.8 (89)	0.8 (12)	4.3 (66)
Summer	1,742	7.7 (134)	6.7 (117)	1.3 (23)	4.4 (76)
Autumn	2,827	8.2 (233)	7.6 (215)	1.7 (48)	4.2 (120)
p†		0.97	0.07	0.55	0.22
Region					
South	2,842	7.4 (211)	6.4 (181)	1.3 (38)	3.7 (105)
Middle	1,920	8.5 (163)	7.7 (148)	1.6 (31)	4.8 (92)
North	1,928	8.5 (163)	6.5 (126)	1.2 (24)	3.7 (72)
Scotland	711	8.2 (58)	7.2 (51)	2.0 (14)	4.9 (35)
p†		0.24	0.54	0.49	0.33
Socioeconomic Position*					
I or II	2,998	6.1 (182)	6.1 (183)	0.9 (27)	3.0 (89)
IIINM	1,520	8.8 (133)	7.6 (115)	1.3 (20)	4.3 (66)
IIIM	1,416	8.1 (187)	5.9 (136)	1.5 (34)	4.3 (100)
IV or V	1,152	16.2 (93)	12.5 (72)	4.5(26)	8.5 (49)
other/unknown	315				
p†		<0.001	0.002	<0.001	<0.001
Physical Activity*					
<2-3 x per month	2,393	9.7 (233)	8.4 (200)	1.9 (45)	4.9 (118)
1 x per week	1,349	6.3 (85)	5.0 (67)	1.0 (13)	2.4 (32)
2-3 x per week	1,552	7.0 (108)	5.8 (90)	1.1 (17)	3.4 (53)
4-7 x per week	1,881	7.6 (142)	6.7 (126)	1.5 (28)	4.9 (92)
Missing	226	12.0 (27)	10.2 (23)	1.8 (4)	4.0 (9)
p†		0.006	0.02	0.20	0.86

	Total (n=7,401)	Depression (n=595) % (n)	Anxiety (n=506) % (n)	Panic (n=107) % (n)	Phobia (n=304) % (n)
Smoking status*					
Never smoked	3,409	7.0 (240)	6.2 (212)	1.2 (41)	2.8 (94)
Ex-smoker	2,027	7.2 (146)	5.6 (113)	0.9 (19)	3.8 (77)
Smokes 1-19	861	8.4 (72)	8.6 (74)	2.4 (21)	6.3 (54)
Smokes ≥ 20	870	12.8 (111)	9.7 (84)	2.5 (22)	8.1 (70)
Missing	234	11.1 (26)	9.8 (23)	1.7 (4)	3.9 (9)
p†		<0.001	<0.001	<0.001	<0.001
Alcohol consumption					
Non-drinker	457	16.4 (75)	11.6 (53)	4.2 (19)	8.3 (38)
Light <7 units per week	3,562	8.0 (285)	6.7 (237)	1.4 (51)	4.0 (141)
Moderate 7-14 units per week	1,852	5.7 (105)	6.0 (111)	0.7 (12)	3.0 (55)
Heavy 14-21 units per week	827	7.9 (65)	6.8 (56)	1.7 (14)	4.6 (38)
Very heavy >21 units per week	687	9.2 (63)	7.1 (49)	1.6 (11)	4.5 (31)
Missing	16	12.5 (2)	-	-	6.3 (1)
p†		0.08	0.81	0.51	0.83
BMI, kg/m^2					
<30	5,628	7.5 (424)	6.6 (370)	1.3 (71)	3.9 (217)
≥30	1,748	9.5 (166)	7.7 (135)	2.0 (34)	4.9 (86)
Missing	25	20.0 (5)	4.0 (1)	8.0 (2)	4.0 (1)
p†		0.007	0.08	0.03	0.04

* Socioeconomic status, physical activity and smoking status taken at 42 years.
† P value from logistic regression trend test adjusted for gender

5.3.2 CMDs and vitamin D-related lifestyles

Certain lifestyle characteristics have been shown to be associated with 25(OH)D concentrations (26) (**Appendix 3.6**). Analyses conducted indicate that symptoms of CMDs were also found to be associated with some but not all of the vitamin D-related lifestyle factors examined. Compared with those without symptoms of CMDs, individuals with depressive, anxiety and phobia symptoms tended to spend more time watching TV or using a PC ($p \leq 0.01$) and participants with anxiety were less likely to seek a suntan ($p = 0.02$) (**Table 5.4)**. When participants did spend time in the sun, individuals with depressive and anxiety symptoms were less likely to use suncover compared to those without CMDs ($p \leq 0.02$). Blistering after sunburn was more likely in those with depressive and panic symptoms compared with individuals with <2 symptoms ($p \leq 0.02$).

Table 5.4: CMDs and vitamin D-related lifestyle factors at 45 years

	Participants (*n*=7,401*)	**>3 h outside/d** (*n*=2,944)	**>3 h TV/PC/d** (*n*=2,376)	**Suncover most of the time** (*n*=6,188)	**Often blister after sunburn** (*n*=129)	**Often actively seek suntan** (*n*=1,391)	**Vitamin D supplement ≥ daily** (*n*=1,185)	**Oily fish at least weekly** (*n*=2,191)	**Margarine at least weekly** (*n*=4,563)
	N	% (n)	% (n)	% (n)	% (n)	% (n)	% (n)	% (n)	% (n)
Depressive symptoms									
< 2 symptoms	6,806	39.9 (2,715)	31.7 (2,155)	84.3 (5,740)	1.6 (110)	19.0 (1,292)	16.2 (1,100)	29.9 (2,037)	62.0 (4,216)
≥ 2 symptoms	595	38.5 (229)	37.1 (221)	75.3 (448)	3.2 (19)	16.6 (99)	14. 2 (85)	25.9 (154)	58.3 (347)
p†		0.88	0.008	<0.001	0.007	0.35	0.48	0.10	0.11
Anxiety symptoms									
< 2 symptoms	6,895	39.9 (2,749)	31.8 (2,191)	83.4 (5,783)	1.7 (115)	19.1 (1,315)	16.0 (1,105)	29.5 (2,034)	61.9 (4,268)
≥ 2 symptoms	506	35.5 (195)	36.6 (185)	80.0 (405)	2.8 (14)	15.0 (76)	15.8 (80)	31.0 (157)	58.3 (295)
p†		0.89	0.01	0.02	0.09	0.02	0.84	0.56	0.19
Panic symptoms									
< 2 symptoms	7,294	39.7 (2,897)	32.0 (2,333)	83.7 (6,105)	1.7 (123)	18.9 (1,378)	16.0 (1,170)	29.5 (2,152)	61.7 (4,503)
≥ 2 symptoms	107	43.9 (47)	40.2 (43)	77.6 (83)	5.6 (6)	12.2 (13)	14.0 (15)	36.5(39)	56.1 (60)
p†		0.19	0.07	0.45	0.02	0.07	0.73	0.09	0.57
Phobia symptoms									
< 2 symptoms	7,097	39.7 (2,815)	31.5 (2,233)	83.7 (5,938)	1.7 (119)	18.8 (1,331)	16.1 (1,142)	29.6 (2,100)	61.8(4,383)
≥ 2 symptoms	304	42.4 (129)	47.0 (143)	82.2 (250)	3.3 (10)	19.7 (60)	14.1 (43)	29.9 (91)	59.2 (180)
p†		0.52	<0.001	0.69	0.08	0.92	0.42	0.85	0.60

* N varies according to missing covariates, ranging from 7,243 (supplements and oily fish data missing) to 6,565 (blistering after sunburn data missing).
† P value adjusted for gender and SEP position at adulthood.

5.3.3 Cross-sectional association

Since some of the vitamin D-related lifestyles were found to be associated with symptoms of CMDs in mid-life, the next step was to examine the association between 25(OH)D and symptoms of CMDs while keeping these potential confounders constant. Results from cross-sectional analysis are shown in **Table 5.5.** It was observed that high 25(OH)D concentrations were associated with lower prevalence of depressive and panic symptoms at 45 years (p_{trend}<0.05, after full adjustment). This association attenuated slightly after adjusting for covariates. Following adjustment for SEP, BMI and lifestyle, participants with 25(OH)D ≥75nmol had 43% (95% CI 19% to 60%) lower odds of depressive symptoms and 67% (25% to 85%) lower odds of panic symptoms compared with those with 25(OH)D <25nmol/l. The association between 25(OH)D and phobia was of marginal significance and there was no significant relationship between 25(OH)D and anxiety symptoms after adjusting for BMI and vitamin D-related lifestyle factors.

A similar pattern emerged after observing the predicted probability of having depressive symptoms at 45 years according to 25(OH)D concentrations (**Figure 5.5**). Here, the predicted probability of having depressive symptoms was lower with higher 25(OH)D concentrations. Additionally, the probability of having depressive symptoms at 25(OH)D ≥75nmol/l was significantly lower compared with 25(OH)D <25nmol/l.

Table 5.5: Association between 25(OH)D and CMDs at 45 years

	25- Hydroxyvitamin D, nmol/l (*n*=7,401)*						
	<25	25-49.9 OR (95% CI)	50-74.9 OR (95% CI)	75-99.9 OR (95% CI)	≥100 OR (95% CI)	p_{trend}	$p_{curvature}$
Depressive symptoms							
Model 1†	1.0	0.64 (0.48, 0.85)	0.50 (0.37, 0.67)	0.43 (0.31, 0.62)	0.32 (0.19, 0.52)	<0.001	0.45
Model 2	1.0	0.68 (0.51, 0 .90)	0.55 (0.40, 0.74)	0.47 (0.33, 0.67)	0.34 (0.21, 0.56)	<0.001	0.76
Model 3	1.0	0.68 (0.51, 0.91)	0.56 (0.41, 0.76)	0.49 (0.34, 0.70)	0.36 (0.22, 0.60)	<0.001	0.78
Model 4	1.0	0.75 (0.56, 1.00)	0.65 (0.48, 0.89)	0.59 (0.41, 0.86)	0.43 (0.26, 0.73)	0.001	0.77
Anxiety symptoms							
Model 1†	1.0	0.80 (0.58, 1.12)	0.64 (0.45, 0.90)	0.67 (0.45, 0.98)	0.68 (0.42, 1.10)	0.03	0.10
Model 2	1.0	0.82 (0.59, 1.14)	0.66 (0.47, 0.94)	0.69 (0.47, 1.02)	0.71 (0.44, 1.15)	0.05	0.14
Model 3	1.0	0.82 (0.59, 1.15)	0.68 (0.48, 0.96)	0.71 (0.48, 1.05)	0.74 (0.45, 1.20)	0.09	0.14
Model 4	1.0	0.94 (0.67, 1.32)	0.85 (0.59, 1.22)	0.97 (0.64, 1.46)	1.03 (0.62, 1.71)	0.98	0.49
Panic symptoms							
Model 1†	1.0	0.42 (0.24, 0.75)	0.38 (0.2, 0.68)	0.19 (0.08, 0.43)	0.20 (0.07, 0.62)	<0.001	0.04
Model 2	1.0	0.46 (0.26, 0.83)	0.44 (0.24, 0.79)	0.21 (0.09, 0.49)	0.23 (0.08, 0.72)	0.001	0.12
Model 3	1.0	0.47 (0.26, 0.84)	0.46 (0.25, 0.85)	0.23 (0.09, 0.54)	0.26 (0.08, 0.81)	0.002	0.12
Model 4	1.0	0.53 (0.29, 0.97)	0.59 (0.31, 1.11)	0.32 (0.13, 0.79)	0.39 (0.12, 1.28)	0.048	0.22
Phobia symptoms							
Model 1†	1.0	0.62 (0.42, 0.92)	0.57 (0.38, 0.86)	0.52 (0.33, 0.84)	0.17 (0.07, 0.41)	<0.001	0.75
Model 2	1.0	0.65 (0.44, 0.96)	0.62 (0.41, 0.92)	0.56 (0.34, 0.90)	0.18 (0.07, 0.44)	<0.001	0.58
Model 3	1.0	0.65 (0.44, 0.97)	0.63 (0.42, 0.94)	0.58 (0.36, 0.93)	0.19 (0.08, 0.46)	<0.001	0.56
Model 4	1.0	0.75 (0.50, 1.12)	0.81 (0.53, 1.23)	0.77 (0.47, 1.28)	0.25 (0.10, 0.62)	0.052	0.22

* Multiple imputation used for missing information on covariates.

†Model 1 was adjusted for gender & season. Model 2 was adjusted for gender, season & socioeconomic position. Model 3 was adjusted for gender, season & socioeconomic position and Body Mass Index. Model 4 was adjusted for gender, season, socioeconomic position, Body Mass Index, smoking and physical activity, PC/TV leisure time, suncover, blistering after sunburn, actively seeking suntan.

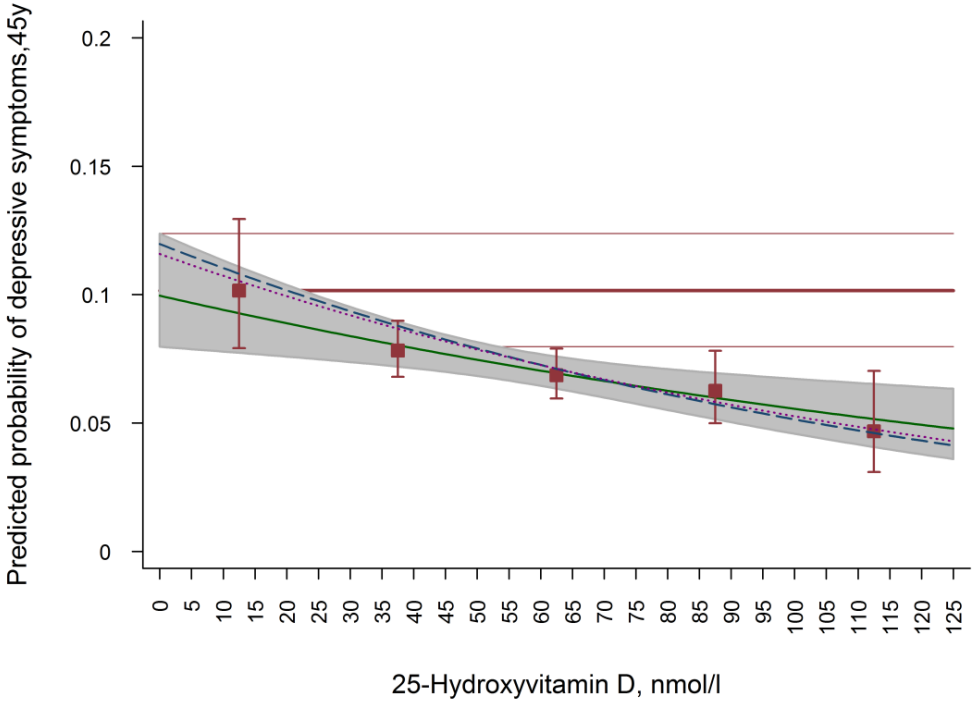


Figure 5.5: Predicted probability of depressive symptoms according to 25(OH)D concentrations at 45 years

N= 7,401. Values are probability (95% Prediction Interval; PI) of having depression at 45 for fully adjusted models. Purple dot line adjusted for gender, season and socioeconomic position (SEP). Navy dash line adjusted for gender, season, SEP and BMI. Green solid line adjusted for gender, season, SEP, BMI, smoking, physical activity, TV/PC time, actively seeking suntan, blistering after sunburn and use of suncover. Shaded areas show 95% PI for fully adjusted models.

5.3.4 Prospective association

Following examination of the prospective association between categorical 25(OH)D at 45 years and depressive symptoms at 50 years (**Table 5.6**), it appeared that a non-linear relationship may be more appropriate. Despite some attenuation after adjustment for BMI and vitamin D related behaviours, a significant non-linear association remained ($p_{curvature}$=0.02).

Data remained supportive of a non-linear relationship following investigation of the predicted probability of having depressive symptoms at 50 years according to 25(OH)D concentrations at 45 years. As seen in **Figure 5.6**, the probability of having depressive symptoms at 50 years was lower for participants with 25(OH)D concentrations between 50 and 85nmol/l compared to those with lower or higher concentrations.

Table 5.6: Association between 25(OH)D and depressive symptoms at 50 years

	25- Hydroxyvitamin D, nmol/l (*n*=5,966)*						
	<25	25-49.9 OR (95% CI)	50-74.9 OR (95% CI)	75-99.9 OR (95% CI)	≥100 OR (95% CI)	P_{trend}	$P_{curvature}$
Depressive symptoms (50 years)							
Model 1	1.0	0.71 (0.53, 0.94)	0.59 (0.44, 0.79)	0.58 (0.41, 0.81)	0.57 (0.37, 0.87)	0.002	<0.001
Model 2	1.0	0.79 (0.54, 0.97)	0.91 (0.45, 0.82)	0.60 (0.43, 0.85)	0.60 (0.39, 0.91)	0.004	0.001
Model 3	1.0	0.78 (0.58, 1.05)	0.67 (0.49, 0.91)	0.67 (0.47, 0.95)	0.17 (0.46, 1.10)	0.044	0.001
Model 4	1.0	0.79 (0.59, 1.07)	0.70 (0.51, 0.96)	0.72 (0.50, 1.02)	0.78 (0.50, 1.21)	0.150	0.002
Model 5	1.0	0.88 (0.65, 1.19)	0.83 (0.60, 1.14)	0.89 (0.61, 1.23)	0.96 (0.61, 1.51)	0.835	0.02

*Multiple imputation used for missing information on covariates. N=5,966

Model 1 was adjusted for gender & season. Model 2 was adjusted for gender, season & socioeconomic position. Model 3 was adjusted for gender, season & socioeconomic position and presence of CMDS at 45 years. Model 4 was adjusted for gender, season, socioeconomic position presence of CMDs at 45 years and Body Mass Index, Model 5 was adjusted for gender, season, socioeconomic position presence of CMDS at 45 years, BMI, smoking and physical activity, PC/TV leisure time, suncover, blistering after sunburn, actively seeking suntan.

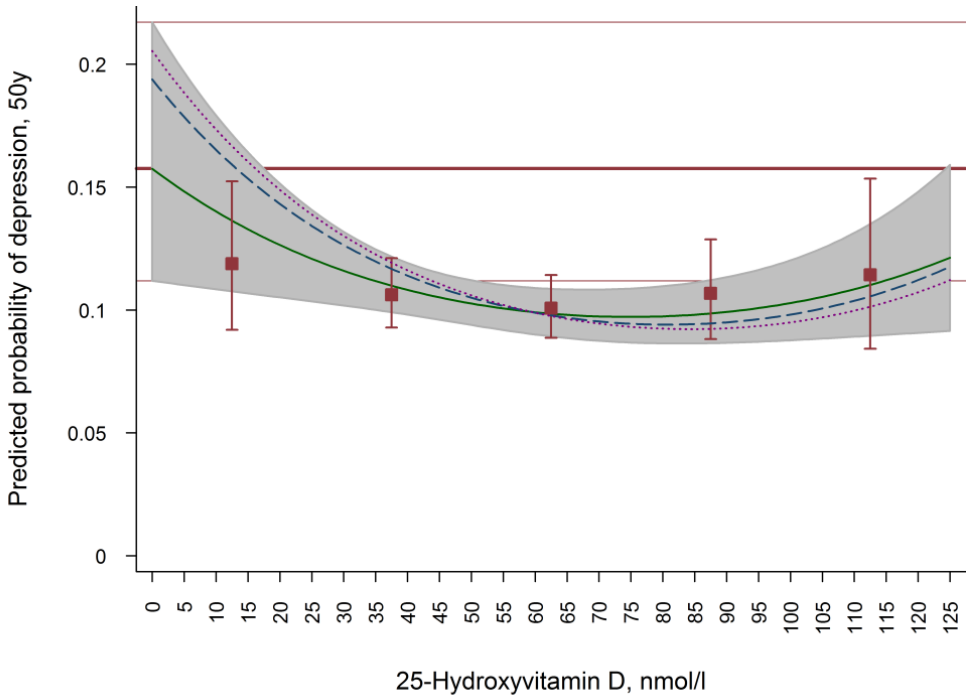


Figure 5.6: Predicted probability of depressive symptoms at 50 years according to 25(OH)D concentrations at 45

N=5,966. Values are probability (95% Prediction Interval; PI) of having depressive symptoms at 50 years for fully adjusted models. Purple dot line, adjusted for gender, season, SEP and symptoms of any CMDs at 45 years. Navy dash line adjusted for gender, season, SEP, presence of any CMDS at 45 years and BMI. Green solid line adjusted for gender, season, SEP, symptoms of any CMDs at 45 years, BMI, smoking, physical activity, TV/PC leisure time, actively seeking suntan, blistering after sunburn and use of suncover. Shaded areas show 95% PI for fully adjusted models.

5.3.5 Additional analysis

To explore the possibility of reverse causality, the association between symptoms of CMDs (as the exposure) and naturally log-transformed 25(OH)D (as the outcome) was examined. The estimates from this analysis were multiplied by 100 to reflect percentage change in 25(OH)D for presence of CMDS (346). As seen in **Table 5.8**, depressive and panic symptoms were associated with slightly lower 25(OH)D concentrations.

Sensitivity analysis was conducted to explore if results differed using a less stringent cut-off for depressive symptoms at 50 years (**Table 5.7**).

Table 5.7: Distribution of different MHI-5 thresholds at 50 years in 1958BC

MHI-5 thresholds	N	(%)
≤52	725	(12.15)
≤60	1,196	(20.05)
≤75	2,222	(37.24)

The overall significant prospective association between 25(OH)D and depressive symptoms remained despite using different thresholds of MHI-5 (**Table 5.9**). However, the protective association between 25(OH)D and depressive symptoms was found to be slightly stronger when using less restrictive thresholds for MHI-5 depressive symptoms.

A comparison of complete-case, weighted-case and imputed datasets is given in **Table 5.10**.

Table 5.8: Association between CMDs and 25(OH)D at 45 years

	N*	Coefficient (95% CI), % change in 25(OH)D	P_{trend}
Depressive symptoms			
Model 1†	7401	-10.15 (-13.74, -6.55)	<0.001
Model 2	7401	-9.28 (-12.88, -5.67)	<0.001
Model 3	7401	-8.36 (-13.10, -4.82)	<0.001
Model 4	7401	-5.47 (-8.88, -2.07)	0.002
Anxiety symptoms			
Model 1†	7401	-4.78 (-8.66, -0.90)	0.02
Model 2	7401	-4.31 (-8.19, -0.42)	0.03
Model 3	7401	-3.78 (-7.60, 0.04)	0.05
Model 4	7401	-0.09 (-3.77, 3.59)	0.96
Panic symptoms			
Model 1†	7401	-19.71 (-27.92, -11.51)	<0.001
Model 2	7401	-18.24 (-26.46, -10.04)	<0.001
Model 3	7401	-16.41 (-24.50, -8.32)	<0.001
Model 4	7401	-11.01 (-18.80, -3.22)	0.01
Phobia symptoms			
Model 1†	7401	-9.82 (-14.75, -4.89)	<0.001
Model 2	7401	-9.04 (-13.97, -4.11)	<0.001
Model 3	7401	-8.24 (-13.10, -3.38)	0.001
Model 4	7401	-4.20 (-8.87, 0.47)	0.08

* Multiple imputation used for missing information on covariates.

†Model 1 was adjusted for gender & season. Model 2 was adjusted for gender, season & socioeconomic position. Model 3 was adjusted for gender, season & socioeconomic position and Body Mass Index. Model 4 was adjusted for gender, season, socioeconomic position, Body Mass Index, smoking and physical activity, PC/TV leisure time, suncover, blistering after sunburn, actively seeking suntan.

Table 5.9: Association between 25(OH)D and depressive symptoms (MHI-5 ≤60 and ≤75) at 50 years

		25- Hydroxyvitamin D, nmol/l*						
		<25	25-49.9 OR (95% CI)	50-74.9 OR (95% CI)	75-99.9 OR (95% CI)	≥100 OR (95% CI)	P_{trend}	$P_{curvature}$
MHI-5 ≤60								
	Model 1†	1.0	0.72 (0.67, 0.78)	0.55 (0.51, 0.59)	0.55 (0.50, 0.60)	0.49 (0.43, 0.54)	< 0.001	< 0.001
	Model 2	1.0	0.74 (0.69, 0.80)	0.57 (0.53, 0.62)	0.57 (0.52, 0.62)	0.50 (0.45, 0.56)	< 0.001	< 0.001
	Model 3	1.0	0.79 (0.73, 0.86)	0.62 (0.57, 0.67)	0.62 (0.57, 0.68)	0.59 (0.52, 0.66)	< 0.001	< 0.001
	Model 4	1.0	0.80 (0.74, 0.86)	0.63 (0.58, 0.69)	0.64 (0.59, 0.71)	0.62 (0.55, 0.70)	< 0.001	< 0.001
	Model 5	1.0	0.88 (0.82, 0.96)	0.74 (0.68, 0.80)	0.77 (0.70, 0.85)	0.74 (0.65, 0.83)	< 0.001	< 0.001
MHI-5 ≤75								
	Model 1†	1.0	0.78 (0.73, 0.84)	0.66 (0.62, 0.71)	0.63 (0.58, 0.68)	0.63 (0.58, 0.70)	< 0.001	< 0.001
	Model 2	1.0	0.80 (0.75, 0.86)	0.69 (0.64, 0.73)	0.65 (0.60, 0.70)	0.65 (0.59, 0.71)	< 0.001	< 0.001
	Model 3	1.0	0.84 (0.79, 0.90)	0.73 (0.68, 0.78)	0.70 (0.64, 0.75)	0.73 (0.66, 0.81)	< 0.001	< 0.001
	Model 4	1.0	0.85 (0.79, 0.91)	0.74 (0.69, 0.80)	0.71 (0.66, 0.77)	0.76 (0.69, 0.83)	< 0.001	< 0.001
	Model 5	1.0	0.91 (0.85, 0.98)	0.84 (0.79, 0.91)	0.83 (0.76, 0.90)	0.88 (0.79, 0.97)	< 0.001	< 0.001

*Multiple imputation used for missing information on covariates. *N*=5,966

†Model 1 was adjusted for gender & season. Model 2 was adjusted for gender, season & socioeconomic position. Model 3 was adjusted for gender, season & socioeconomic position and presence of CMDS at 45 years. Model 4 was adjusted for gender, season, socioeconomic position presence of CMDs at 45 years and Body Mass Index, Model 5 was adjusted for gender, season, socioeconomic position presence of CMDs at 45 years, BMI, smoking and physical activity, PC/TV leisure time, suncover, blistering after sunburn, actively seeking suntan.

Table 5.10: Comparison of complete, weighed and imputed cross-sectional and prospective results

	25- Hydroxyvitamin D, nmol/l					p_{trend}	$p_{curvature}$
	<25	25-49.9 OR (95% CI)	50-74.9 OR (95% CI)	75-99.9 OR (95% CI)	≥100 OR (95% CI)		
Cross-sectional analysis with depressive symptoms at 45 years							
Complete-case results (n=6,132)	1.0	0.73 (0.52, 1.03)	0.67 (0.46, 0.96)	0.59 (0.38, 0.91)	0.40 (0.22, 0.74)	0.003	0.35
Weighted-case results (n=5,935)	1.0	0.76 (0.53, 1.08)	0.69 (0.47, 1.00)	0.59 (0.38, 0.93)	0.43 (0.23, 0.80)	0.01	0.41
Imputed results (n=7,401)	1.0	0.75 (0.56, 1.00)	0.65 (0.48, 0.89)	0.59 (0.41, 0.86)	0.43 (0.26, 0.73)	0.001	0.77
Prospective analysis with depressive symptoms at 50 years							
Complete-case results (n=5095)	1.0	0.90 (0.64, 1.26)	0.88 (0.61, 1.25)	0.92 (0.61, 1.38)	1.13 (0.68, 1.85)	0.69	0.03
Weighted-case results (n=4937)	1.0	0.90 (0.64, 1.27)	0.87 (0.61, 1.25)	0.85 (0.56, 1.28)	1.09 (0.66, 1.83)	0.99	0.02
Imputed results (n=5,966)	1.0	0.88 (0.65, 1.19)	0.83 (0.60, 1.14)	0.89 (0.61, 1.23)	0.96 (0.61, 1.51)	0.84	0.02

Cross-sectional results adjusted for gender, season, socioeconomic position, Body Mass Index, smoking and physical activity, PC/TV leisure time, suncover, blistering after sunburn, actively seeking suntan. Prospective results additionally adjusted for presence of any CMDs at baseline

5.4 Discussion

The aim of this chapter was to explore the relationship between 25(OH)D and CMDs using methods of observational epidemiology. A paper related to these findings has been published in the peer-reviewed journal, *Clinical Nutrition* (347) and can be found in **Appendix 1.1**.

The presence of a CMD can lead to changes in lifestyle that affect the health of the individual, such as, an increase in cigarette smoking (348), yet, little is known about alterations to specific vitamin D-related behaviours. Results indicated that some, but not all vitamin D-related lifestyle factors differ between participants with and without symptoms of CMDs. Findings suggest that those with symptoms of CMDs may spend more time watching TV/PC, which could imply a less active and indoor lifestyle resulting in reduced 25(OH)D concentrations. Those with CMDs also reported harmful sun-exposure behaviours such as blistering after sunburn and rarely using suncover which have both been associated with reduced 25(OH)D concentrations. Cross-sectional analyses found a significant, moderate linear association between low 25(OH)D concentrations and prevalence of depressive and panic symptoms remained, despite adjustment for vitamin D-related lifestyles. Here, participants with 25(OH)D <25nmol/l were found to have higher odds of depressive or panic symptoms compared with those with ≥75nmol. To extend these results, prospective analyses were conducted and a significant non-linear association between 25(OH)D and depressive symptoms was demonstrated. The odds of having depressive symptoms were lower for participants with 25(OH)D concentrations between 50 and 85nmol/l compared to those with lower or higher concentrations.

Comparison between findings from this study and results from previous work is difficult due to the variety of study designs, populations and instruments used to assess CMDs. However, results are consistent with a meta-analysis (184) that reported a significant inverse association between 25(OH)D and CMDs using both cross-sectional and prospective designs.

There are many potential variables that can confound the relationship between 25(OH)D and CMDs (**Chapter 1**). This study had the unique inclusion of vitamin D-related lifestyles which have not been assessed to a great extent in previous work. The discovery that these vitamin D-related lifestyles are different amongst those with and without symptoms of CMDs emphasise their potential importance on the relationship between 25(OH)D and CMDs. In effect, findings imply that the association between 25(OH)D and CMDs may not be solely dependent on vitamin D-related behavioural traits.

Most former cross-sectional studies examining the association between 25(OH)D and CMDs have focused on depressive symptoms (**Table 5.1**). The potential mechanisms through which active vitamin D have been proposed to affect brain function (discussed **Chapter 1**) are relatively broad and therefore may not be unique in their influences on depressive symptoms. Hence, all available categories of CMDs, i.e. depressive, anxiety, panic and phobia symptoms were examined. The relationship found between 25(OH)D and depressive symptoms is in line with the literature and may be related to the effect of active vitamin D (i.e. $1,25(OH)_2D$) on certain neurotransmitters, such as serotonin, involved in the development of depression (349). The association of 25(OH)D with panic symptoms is less well documented. The relationship observed in the current study may be due to specific biological mechanisms affected by 25(OH)D concentrations, possibly related to the overarching physical symptoms of the disorder. However, a spurious relationship due to low numbers of individuals affected by panic in the sample (1.5%) cannot be ruled out. The lack of association noted between 25(OH)D and anxiety or phobia may point to an aetiology that is truly independent of an influence of 25(OH)D, or again, may have been unduly dominated by methodological limitations. CMD type-specific analyses might be used to provide insights into possible mechanisms, however, these would need to be interpreted with caution, given limitations of our mental health measures, comorbidity between different symptoms and the limited numbers of participants presenting with panic and phobia.

The present work provides a temporal aspect to the research on 25(OH)D and CMDs by conducting one of few prospective studies on 25(OH)D and

depressive symptoms. Other prospective investigations (334, 336) demonstrating a protective effect of higher 25(OH)D concentrations on depressive symptoms were consistent with the current reported results. However, Chan et al. did not replicate this finding (335). An association was observed between 25(OH)D and less severe depressive symptoms was identified during sensitivity analyses. This finding could imply that the relationship between 25(OH)D and depressive symptoms may be most beneficial for participants with relatively mild depressive symptoms compared with those with severe depressive symptoms. Therefore the association between 25(OH)D and a clinical diagnosis of depression may be different (**Table 5.1)**.

Results from the small number RCTs examining the effect of vitamin D supplementation on CMDs have been inconsistent (341, 350), therefore the direction of the association remains unknown. It is not possible to ascertain causality using a cross-sectional study design. While the prospective study provides some temporal aspect to the relationship between 25(OH)D and depressive symptoms, causality remains uncertain. Results from sensitivity analyses, where 25(OH)D was treated as the dependent variable, suggest that depressive and panic symptoms were associated with a small reduction in 25(OH)D concentrations. This indicates that the possibility of reverse causation cannot be ruled out.

One finding that has not, to the best of my knowledge, been examined in previous studies, was the presence of a non-linear prospective relationship between 25(OH)D and depressive symptoms. The finding of a non-linear association in prospective analyses has a number of potential explanations. The non-linear relationship between 25(OH)D and CMDs may be indicative of a threshold effect, whereby increasing 25(OH)D concentrations may only be beneficial up to levels of approximately 75nmol/l. Any increases in 25(OH)D beyond this threshold could have either no effect on reducing depressive symptoms or may even be harmful. Conversely, there could be an underlying biological explanation for the non-linear association between 25(OH)D and CMDs. In a study showing associations of low and high 25(OH)D with higher risk of prostate cancer (351), the investigators suggested that these two levels

of 25(OH)D may exert their effects though different mechanisms. They argued that high concentrations may affect vitamin D metabolism leading to an increase in 24-hydroxylation. In the presence of very high 25(OH)D concentrations, this process degrades the active form of vitamin D (i.e. 1,25(OH)D_2) resulting in reduced bioavailability of 1,25(OH)D_2 in the tissue. If this was the case, it could be speculated that high 25(OH)D concentrations resulted in lower 1,25(OH)D_2, reducing stimulation of the VDR receptor in the brain. This non-linear relationship could have implications for determining the most beneficial 25(OH)D threshold to protect against depressive symptoms, however further replication is required.

Results from this study should be interpreted with methodological limitations in mind. Comparison between compete-case to weighted-case and imputed results showed no difference, thereby increasing confidence that the observed estimates were not affected by attrition or missing data **(Chapter 3)**. However, the use of different instruments to measure CMDs at 45 and 50 years **(Chapter 4)** may have affected the ability to control for CMDs at 45 years in the prospective analyses by not being directly comparable. Furthermore, responses to lifestyle questions may be dependent on the participant's mood. This phenomenon is known as the response set effect, whereby individuals who have a CMD may have a tendency to answer a question in a negative way, which could have an effect of the validity of the questionnaire data. (352).

The aetiology of both CMDs and vitamin D deficiency are complex and their association contains a multitude of confounding factors. Although this study had the advantage of applying a comprehensive statistical adjustment for potential confounders, as with all observational epidemiology, residual confounding may remain. This could be due to lack of information on some confounding factors as well as errors in measurement of covariates, exposures or outcomes.

5.4.1 Conclusion

The high burden of mental and behavioural disorders and concurrent high prevalence of vitamin D insufficiency (<75nmol/l) worldwide (17) highlight the

potential importance of the study findings. Using methods from observational epidemiology, results from this chapter suggest that low 25(OH)D is associated with higher prevalence of depressive and panic symptoms and that 25(OH)D is modestly and non-linearly associated with subsequent depressive symptoms.

5.5 Summary

- There is evidence of a link between hypovitaminosis D and common mental disorder, but studies lack investigation of behaviours relating to vitamin D status, which may explain this association
- The aim of this chapter was to assess the relationship between 25(OH)D and common mental disorder in mid-adulthood adjusting for vitamin D-related lifestyles using a cross-sectional and prospective design
- There was an association between hypovitaminosis D and prevalent depressive symptoms and a non-linear association between 25(OH)D and subsequent depressive symptoms. However, uncertainty regarding the causal nature of the relationship between 25(OH)D and depressive symptoms remains

Chapter 6 Observational study: Association between vitamin D and cognitive function

6.1 Introduction

Observational findings from the previous chapter were supportive of an inverse association between 25-hydroxyvitamin D (25(OH)D) and emotional well-being. As mentioned in **Chapter 1**, there is also some evidence of an association between 25(OH)D and cognitive function including work on biological plausibility. It is this association between 25(OH)D and cognitive function which will be explored for the remainder of the thesis.

Cognitive function changes throughout the life-course from rapid development in early life, to plateau in adulthood and then decline with increasing age (**Chapter 1**). While there is consensus that cognitive decline is evident in older adults (aged ≥65 years), there is some suggestion that decline may begin at an earlier age (104). With this earlier age in mind, work presented in this chapter focuses on the association between 25(OH)D and cognitive function in mid-life (age 50 years). There have been a number of observational studies examining the association of 25(OH)D with cognitive function. To examine the evidence to date, methods of a systematic review were applied on May 8, 2013. Studies relating to vitamin D and cognitive function were identified by searching in PubMed for articles published within the past 10 years (i.e. from 2003) (*n*=2,031). Selected studies were limited to those published in the English language (*n*=1,876) and in human populations (*n*=1,342). Search terms used are illustrated in **Appendix 4.1** There were 60 relevant studies identified following examination of the titles. From the 60, 29 were excluded following inspection of the abstracts (*n*=17 were reviews or commentary, *n*=5 used child or adolescent samples, *n*=7 were deemed irrelevant). Furthermore, reference lists of previous systematic reviews (185, 353, 354) were examined for additional relevant citations (*n*=20), resulting in a total of 51 studies. No full text was available for 5 of the studies; therefore the total number identified was 46. Details of these studies are given in **Table 6.1** which has been adapted from a table used in a previous systematic review (185). Further information, including study setting, groups and adjustments for confounders can be found in **Appendix 4.2**.

Table 6.1: Systematic review: Vitamin D and cognitive function

Author (year), country	Total N	Age (y); Mean ± SD; (min-max)	Female (%)	Vitamin D, 25(OH)D unless stated		Cognitive tests; Dementia Criteria	Results
				Method	Cut-point, nmol/l		
Cross-sectional							
McGrath (2007)(355), USA	11, 232	(12-90)	Unknown	RIA DiaSorin	Q1: <39.5 Q2: 52.9 Q3: 66.9 Q4: 84.9 Q5: >84.9	Mean reaction time Symbol-digit substitution Serial digit learning Memory and learning score	Adult group: *Mean reaction time(SD) n=4,747*: Q1: 229(2.9); Q2:231.2(2.3): Q3:235.4(3.7): Q4:232.9(1.7): Q5:233.7(2.57), *p*=0.60 *Symbol-digit substitution mean(SD)* n=4,688: Q1: 2.62(0.04); Q2:2.65(0.04); Q3:2.63(0.03): Q4:2.64(0.03); Q5:2.69(0.03),*p*=0.4 0 *Serial digit learning mean(SD) n=4,584*: Q1: 4.5(0.1); Q2:4.6(0.1); Q3:4.6(0.1); Q4:4.5(0.1); Q5:4.7(0.1), *p*=0.60 Fully adjusted OR(95%CI) with Q5 as reference for symbol digit substitution (≥2): Q1: 0.75 (0.52,1.09); Q2: 0.67(0.47,0.96); Q3: 1.00(0.72,1.40), Q4: 0.80(0.58, 1.11), *p*=0.11, Fully adjusted OR(95%CI) with Q5 as reference for serial digit learning (≥11): Q1: 1.03 (0.61,1.74); Q2: 1.21(0.69,2.14); Q3: 1.21(0.77,1.90), Q4: 0.99(0.62, 1.58), *p*=0.80, Elderly Group: *memory & learning mean(SD) n=4,809:* Q1: 6.5(0.1); Q2:6.4(0.1); Q3:6.6(0.1); Q4:6.6(0.1); Q5:6.4(0.1), *p*=0.02

Author (year), country	Total N	Age (y); Mean ± SD; (min-max)	Female (%)	Vitamin D, 25(OH)D unless stated		Cognitive tests; Dementia Criteria	Results
El-Ghoneimy (2009)(356), Egypt	30	(18-40)	63.3	ELISA Bioscientia	<50	PASAT FST	*p*-value not significant for all cognitive tests using Pearson correlation
Hansen (2011)(357), Norway	25	(20-60)	0	RIA In-house	<50 ≥50	0-back: non-executive function 2-back:excutive function	2-back mean (SD) *>50nmol/l: 36(3.84) vs. <50nmol/l 29.81(9.55)* p<0.05, Cohen's D=0.85 0-back mean (SD) *>50nmol/l: 38.57(1.02) vs. <50nmol/l* 38.27(1.49) p>0.10, Cohen's D=0.23
Tolppanen (2011)(358), USA	4932	(20-90)	Unknown	RIA DiaSorin	NA	Mean reaction time Symbol-digit substitution Serial digit learning Memory and learning score	Young adults mean difference (95% CI) *Mean reaction time (n=4929):* 0.00 (-0.04 to 0.04) *Symbol digit substitution (n=4869):* 0.00(-0.03 to 0.03) *Serial digit learning (n=4760):* 0.01(-0.05 to 0.06) Older adults mean difference (95% CI) *Recalled items (n=4831):* -0.01(-0.06 to 0.05)
Lee (2009)(359), Italy, Belgium. Poland, Sweden, UK, Spain, Hungary, Estonia	3369	(40-79)	0	RIA DiaSorin	<25 25-<50 50-75 ≥75	ROCF (copy) ROCF (recall) CTRM DSST	ROCF (copy) *Beta (95%CI) per 10nmol/l*: 0.06 (-0.01, 0.14) *Beta (95%CI) compared with ≥75*: 50-74.9: -0.33(-0.73, 0.07); 25-49.9: -0.33 (-0.73, 0.07); *<25:* -0.61 (-0.13, 0.09) ROCF (recall) *Beta (95%CI) per 10nmol/l*: -0.21 (-0.16, 0.12) *Beta (95%CI) compared with ≥75:* 50-74.9:-0.12(-0.57, 0.34); 25-49.9: 0.26 (-0.66, 1.18); <25: -0.46 (-0.37, 0.45) CTRM *Beta (95%CI) per 10nmol/l:* -0.001 (-0.15, 0.14)

Author (year), country	Total N	Age (y); Mean ± SD; (min-max)	Female (%)	Vitamin D, 25(OH)D unless stated		Cognitive tests; Dementia Criteria	Results
							Beta (95%CI) compared with ≥75: 50-74.9:-0.14(-0.75, 0.47); 25-49.9: 0.08 (-0.87, 1.04); <25: -0.13 (-1.3, 1.05) DSST *Beta (95%CI) per 10nmol/l:* 0.15 (0.05, 0.25), *p*<0.01 *Beta (95%CI) compared with ≥75*: 50-74.9:-0.76(-1.31, -0.20), p<0.05; 25-49.9: -0.77 (-1.82, 0.29); <25: -1.40 (-2.68, -0.13), *p*<0.05
Seamans (2010)(360), Ireland, France, Rome, Italy	387	(55-87)	49.4	ELISA IDS	T1: <47.6 T2: 47.6-85.8 T3: >85.8	CANTAB Visual memory working memory attention	SWM between errors Median(IQR): T1 (n=127): 39.0 (21.0,52); T2(n=127): 32.0(13,49); T3(n=126): 27.5(15.0-43.0) Beta(SE): T1 vs. T2: 3.15(2.37, *p*=0.19 T1 vs. T3: 5.39(2.44), *p*=0.03 Females only: T1 vs. T2: 2.87(3.45), *p*=0.41 T1 vs. T3: 5.36(2.60), *p*=0.004 SWM between errors (8 boxes) Median(IQR): T1: 28 (16,35); T2 25(15-31); T3: 22.0(11.0-32) Beta(SE): T1 vs. T2:1.46(1.74), *p*=0.40 T1 vs. T3: 3.14(1.81), *p*=0.08 Females only: T1 vs. T2: -1.05(2.47), *p*=0.67 T1 vs. T3: 5.29(2.58), *p*=0.04

Author (year), country	Total N	Age (y); Mean ± SD; (min-max)	Female (%)	Vitamin D, 25(OH)D unless stated	Cognitive tests; Dementia Criteria	Results
						<u>SWM strategy</u> Median(IQR): T1: 35 (33-38); T2 35(33-38); T3: 33.0(22-36.3) Beta(SE): T1 vs. T2: 1.41(1.00), *p*=0.16 T1 vs. T3: 2.36(1.03), *p*=0.02 Females only: T1 vs. T2: 1.81(1.38), *p*=0.19 T1 vs. T3: 4.73(1.40), *p*=0.001 <u>SWM total errors</u> Median(IQR): T1: 41 (22-55); T2 35(13.8-53); T3: 28.5(15.8-45) Beta(SE): T1 vs. T2: 3.11(2.52), *p*=0.22 T1 vs. T3: 5.36(2.60), *p*=0.04 Females only: T1 vs. T2: 2.06(3.62), *p*=0.57 T1 vs. T3: 10.84(3.69), *p*=0.004
Benge (2009)(361), USA	111	60.5 ± 12.3	82.9	Unknown	WAIS-III COWA Grooved Peg TMT HVLT-R PASAT CWI	CSI (n=39) and no CSI (n=72), *p*-value not reported
Oighara (1990)(362), Japan	60	(63-94)	100	CPBA In-house	Dementia Screening Scale of Hasegawa	<u>Mean 25(OH)D (SD), ng/ml</u> *SDAT (n=22)* 17.7 (8.5) *VTD (n=20)*: 20.7 (8.2) No dementia (n=18): 20.7 (8.2)

Author (year), country	Total N	Age (y); Mean ± SD; (min-max)	Female (%)	Vitamin D, 25(OH)D unless stated		Cognitive tests; Dementia Criteria	Results
Houston (2007)(363), Italy	976	≥65	55.4	RIA DiaSorin	<25 25-50 ≥50	MMSE	Men, mean MMSE (SE) <25nmol/l (n=59) : 23.6(0.5); 22-<50 (n=163): 25.6(0.3): ≥50 (n=213) 25.2(0.3), p_{trend} =0.37 , Women , mean MMSE (SE) <25 (n=156): 23.9(0.3); 22-<50 (n=249): 24.0(0.2): ≥50 (n=136) 25.0(0.3), p_{trend}=0.01
Llewellyn (2009)(364), UK	1766	≥65	59.9	RIA DiaSorin	Q1: 8-30 Q2: 34-44 Q3: 45-65 Q4: 66-170	AMT Cognitive impairment: ≥3 AMT	OR (95%CI) with Q4 as reference fully adjusted: Q3: 1.09(0.64-1.86); Q2 1.43 (0.84-2.42); Q1: 2.28 (1.36-3.83), p_{trend}=0.001
Llewellyn (2011)(365), USA	3325	≥65	55.2	RIA DiaSorin	<25 ≥25-50 ≥50-75 ≥75	Immediate verbal memory Delayed verbal memory orientation attention Impairment: worst 10% of the distribution for combined scores Global cognitive function is sum of all, memory is sum of 4 memory tests	Cognitive impairment OR (95%CI) 50-75: 0.86(0.59-1.26); 25-50: 1.42 (0.96-2.09); <25 0.39(1.49-10.43). p_{trend} =0.017 Memory impairment OR (95%CI) 50-75: 0.59(0.43-0.83); 25-50: 1.18 (0.78-1.81); <25 3.18(1.20-8.44). p_{trend} =0.18
Aung (2006)(366), USA	44	≥65 years	75	RIA DiaSorin	<25	MMSE CDT	MMSE score (SD) <25nmol/l (n=15) 23.27 (4.43); ≥25nmol/l (n=28) 24.82 (4.02), *p*=0.25 <25nmol/l (n=15) 7(47%); ≥25nmol/l (n=28)

Author (year), country	Total N	Age (y); Mean ± SD; (min-max)	Female (%)	Vitamin D, 25(OH)D unless stated		Cognitive tests; Dementia Criteria	Results
							MMSE <24, score (SD) <50nmol/l (n=33) 23.91 (4.0); ≥50nmol/l (n=10) 25.5 (4.7), *p*=0.29 CDT, n(%) <25nmol/l (n=13) 4(31%); ≥25nmol/l (n=28) 6(21), *p*=0.52 CDT(abnormal), n(%) <50nmol/l (n=31) 9(26%); ≥50nmol/l (n=10) 1(10%), *p*=0.96
Buell (2009)(367), USA	1080	(65-99)	75.9	RIA DiaSorin	<25 25-50 ≥50	MMSE NAART WMS-III WMS-III LM TMT WAIS-III block design Matrix reasoning COWA	Beta coefficient (SE), partial correlation value: *Executive function factor (n=931):* 0.01(0.003, 0.13; p=0.001 *Memory function factor (n=931):* -0.001(0.004, -0.002; *p*=0.65 *Attention/procession speed factor (n=931):* 0.01 (0.003), 0.08; *p*=0.03 *TMT A:* <25: 92; 25-50:87; ≥50: 82.4; *p*<0.05 *TMT B:* <25: 92; 25-50:87; ≥50: 82.4; *p*<0.05
Buell (2010)(368), USA	318	(65-99)	72.6	RIA DiaSorin		AD: NINDS-ADRDA VTD: NINDS-AIREN Other: DSM-IV	25(OH)D, ng/ml (SD) *AD* (n=41): 16.9(6.3); *Stroke with dementia* (n=22): 17.8(8.5); *Stroke without dementia* (n=31): 19.4 (7.8); *All dementia* 16.8(6.9); *No stroke no dementia*(n=211): 20.0 (8.2) Fully adjusted all-cause dementia (n=70) Beta (SE): 0.79 (0.34), *p*=0.02 OR (95%CI): 2.21(1.13-4.32) Fully adjusted Alzheimer's Disease (n=37) Beta (SE): 0.98 (0.51), *p*=0.05 OR (95%CI): 2.65(0.99-7.16)

Author (year), country	Total N	Age (y); Mean ± SD; (min-max)	Female (%)	Vitamin D, 25(OH)D unless stated		Cognitive tests; Dementia Criteria	Results
Annweiler (2012)(369), France	125	73.4±6.9	58.4	RIA Incstar	Q1: 10-40 Q2:41-59 Q3:60-79l Q4:80-189	MCI: neuropsychological tests, physical examination &MRI scan	CHI(n=52) vs. MCI (n=43) *Mean 25OHD(SD):* 70.6(34.2) vs. 53.5(21.8), p=0.006 q1 n(%): 10(19.2) vs. 14(32.6) q2: 13(25) vs. 13(30.2) q3: 11(21.2) vs. 12(27.9) q4: 18(34.6) vs. 4(9.3), *p*=0.03. OR(95%CI) Fully adjusted (MCI), q4 reference q3: 10.24(1.22-85.94), p=0.03 q2: 7.37(1.16-46.87), p=0.03 q1: 49.64(4.62-533.28), p=0.001 Stepwise backwards OR(95%CI) Fully adjusted (MCI), q4 reference q3: 10.29(1.38-76.75), *p*=0.02 q2: 6.89(1.17-40.52), *p*=0.03 q1: 25.46(3.22-201.28), *p*=0.002, OR(95%CI) of MCI 0.96(0.93-0.98), *p*=0.002
Wilkins (2006)(324), USA	80	74.8±7.7	62.5	RIA DiaSorin	<25 25-50 ≥50	MMSE SBT CDR sum of boxes Factor score	MMSE, score (SD): ≥*50*:26.12(3.46); *25-50*: 26.06(4.11); <*25*:24.77 (4.32), *p*=0.53 SBT, score (SD): ≥*50*:4.15(4.75); *25-50*:7.42(4.80); <*25*: 9.92(8.94), *p*=0.01 Factor, score (SD): :≥*50*:-1.01(1.56); *25-50*:-1.35 (1.60); <*25*: -0.59(1.96), *p*=0.52

Author (year), country	Total N	Age (y); Mean ± SD; (min-max)	Female (%)	Vitamin D, 25(OH)D unless stated		Cognitive tests; Dementia Criteria	Results
							CDR, score (SD): ≥50:1.65(2.08); 25-50: 2.36 (2.56), <25 3.08(3.07) *p*=0.04 Dementia (CDR>0) Adjusted OR(95%CI): <25 versus ≥50: 2.80(0.64,12.28) 25-50 versus ≥50:1.78(0.61,5.19)
Wilkins (2009)(370), USA	60	75±8.2	N/A	RIA DiaSorin	<50 ≥50	MMSE SBT	MMSE, score (SD): <50 25.39(3.9); ≥50: 25.07 (4.9), *p*=0.78 SBT, score (SD): <50:10.87(7.5); ≥50: 6.31(6.7), *p*=0.016 SBT: Adjusted Beta(SE), 2.51(1.17), *p*=0.36
Annweiler (2010)(371), France	752	≥75	100	RIA DiaSorin	<25	SPMSQ Cognitive impairment: (SPMSQ <8)	SPMSQ score (SD) ≥10ng/mL (n=623): 9.05 (1.34);<10ng/mL (n=129) 8.56 (1.67), *p*<0.001 Adjusted Beta coefficient (95% CI) -0.003 (-0.012, 0.006), *p*=0.512 Adjusted OR (95%CI) 1.99 (1.13, 3.52), *p*=0.017, logistic regression 2.03 (1.17, 3.53), *p*=0.012, stepwise backward
Oudshoorn (2008)(372), Netherlands	225	77.6±7.3	65	RIA DiaSorin	<50 ≥50	MMSE	MMSE <50nmol/l (n=141); ≥50nmol/l (n=84) Fully adjusted Beta (95%CI): 0.05 *p*=0.01
Perez-Llamas(2008)(373), Spain	86	77.4±8.1	66.3	HPLC CIC	<50 ≥50	SPMSQ	SPMSQ , score (SD) <50(n=50): 7.9(2.0); ≥50(n=36): 7.7 (2.0), *p*=0.70
Przybelski (2007)(374), USA	80	79.5±1.6	N/A	RIA DiaSorin	<25 25-50 ≥50	MMSE	Pearson correlation r^2=0.225, *p*=0.006

Author (year), country	Total N	Age (y); Mean ± SD; (min-max)	Female (%)	Vitamin D, 25(OH)D unless stated		Cognitive tests; Dementia Criteria	Results
Skalska (2012)(375) Poland	140	79.67±6.99	67.10%	EIA IDS	T1: <23.26 T2: 23.26-47.75 T3: >47.75	AMT cognitive impairment: AMT <7	AMT, mean (SD): T1: 704(3); t2: 6.9(3): T3: 8.4(2) T1 vs. t2: p=0.78 ; T2 vs. T3: 0.03 ; T1 vs. T3: *p*=0.15 OR(95%CI) T2 vs. T1: 3.17(1.04-9.67) *p*=0.04
Annweiler (2010)(376), France	5596	80.5 ± 0.1	100	FFQ (dietary intake)	<35µg/wk ≥35µg/wk	SPMSQ Cognitive impairment: (SPMSQ <8)	SPMSQ mean (SD) ≥35µg/wk (n=4802): 8.97 (1.24); <35µg/wk(n=794) 8.80 (1.31), *p*<0.001 Adjusted Beta coefficient (95%CI) 0.001 (0.001, 0.002), p<0.024 Adjusted OR(95%CI) 1.30 (1.04, 1.63), *p*=0.24
Sakuma (2006)(377), Japan	50	82.6 ±8.7	82%	ELISA DiaSorin		Dementia severity criteria developed by the Japanese Ministry of Health, Labour and Welfare, 1993	Serum 25(OH)D decreased as dementia progressed *Cognitively normal*: 53.7(17.5), *p*<0.05
Annweiler (2011)(378), France	228	86±0.4	100		<25 ≥25	MMSE	Severe dementia % <10ng/ml (n=138): 42.5 >10: 25.2, *p*=0.1, Fully adjusted moderate, severe dementia OR(95%CI) 2.57(1.05-6.27), *p*=0.04 Step-wise backward moderate, severe dementia OR(95%CI) 2.39(1.12-5.09), *p*=0.02
Ravaglia (1998)(379), Italy	27	97.6 ±3.2		RIA for 1,25OHD		DSM-III	Mean (SD), 1,25OHD concentration in pmol/L: *Dementia* (n=15): 43.2 (20.16) *No dementia* (n=12): 72.72(18.96) *p*=0.009

Author (year), country	Total N	Age (y); Mean ± SD; (min-max)	Female (%)	Vitamin D, 25(OH)D unless stated		Cognitive tests; Dementia Criteria	Results
Case-control							
Evatt (2008)(380), USA	196	AD (47-88); Controls (39-89)	43; 43	ELISA IDS	NA	NINCDS-ADRDA (AD)	Mean ng/ml(SD) AD: 86.86(15.4); Control: 37(14.5), *p*=0.30
Martyn (1989)(381), UK	61	(58-90); (66-89)	100 100	RIA Unknown	NA	Intellectual deterioration over a period of more than 6 months and no other indication of dementia	Mean ug/ml AD (n=27): 11.5; Control(n=34):14.3 Difference (95%CI) -2.8(-4.9 to -0.7), *p*<0.05
Walker (2009)(382), USA	128	61.3±1.0 55.6±0.4	100 100	RIA DiaSorin	NA	Book category NART Rosen target detection RVDLT Serial digit learning WAIS-R digit symbol WAIS digit span WMS logical memory	For all cognitive tests (details not presented): no linear associations observed between any baseline abnormal cognitive variable and 25(OH)D no relationship between changes in cognitive variables and change in 25(OH)D.
Jorde (2006)(333), Norway	84 (148 total cohort)	62.3±15.3; 63.5±13.2	42.8; 38.0	RIA DiaSorin	NA	Digit span forward & backward Seashore Rhythm TMT A and B Stroop test Digit symbol test CalCAP CVLT	Beta coefficient (t value) (from total cohort (n=148) *Digit span forward*: 0.17 (1.39) *Seashore Rhythm*: -0.13(-0.90) *TMT A*: 0.18(1.57) *Stroop test, 1 and 2*: 0.12(1.05) *Digit Symbol test:* -0.06(-0.74) *CalCAP:* 0.04 (0.32)

Author (year), country	Total N	Age (y); Mean ± SD; (min-max)	Female (%)	Vitamin D, 25(OH)D unless stated		Cognitive tests; Dementia Criteria	Results
						Verbal recall Visual recall COWA WAIS	*Verbal recall:* 0.08(0.69) *Visual recall*: (0.13(1.21) *Word list test:* -0.03(-0.26) *COWA*: 0.13(1.08) *Stroop test, 3*: -0.07(-0.68) *TMT B*: -0.06(-0.51) *WAIS*: 0.04(0.31) All t values not significant
Luckhaus (2009)(383), Germany	47	70.4±8.2; 67.3±10.9; 72.4±6.1	40; 47; 50	ELISA IDS	NA	NINCDS-ADRDA (AD)	Mean nmol/ml (SD) AD (n=20): 39.5(24.5); MCI (n=19) 48(18) Control (n=8): 36(13), *p*>0.05 for all
Ferrier (1990)(384), UK	50	76±7 74±5	76.9 75	RIA in-house	NA	DSM-III MTS score <30 HII score <7	Mean nmol/ml(SD) ATD(n=15): 32(9); Control(n=11): 40(25), p=0.30, p>0.05 Mean pg/ml(SD) ATD(n=15): 22(11); Control(n=14): 20(8.4), p=0.30, *p*>0.05
Sato (2005)(385), Japan	200	79.7±4.5; 79.9±5.2; 80.6±6.8	100; 100; 100	CPBA Nichols	NA	DSM-III-R MMSE	Mean nmol/l(SD) Severe AD: 25(10); Mild AD: 40.5(10): Controls 60.5 (11), *p*<0.001
Sato (1998)(386), Japan	186	81.3±5.4; 80±4.7	100 100	CPBA Nichols	NA	DSM-III-R NINDS-ADRDA	Mean nmol/l(SD) AD: 17.7(9.7); Controls 53.9 (7.7), p<0.001
Cohort							
Chan (2011)(335), China	939	>65	0	RIA DiaSorin	Q1: ≤63 Q2: 64-76 Q3: 77-91 Q4: ≥92	CSI-D Cognitive impairment: CSI-D ≤28.4	Adjusted OR (95& CI) for cognitive impairment with Q1 as reference: Q2:0.88 (0.37-2.08); Q3: 0.81(0.32-2.03); Q4: 1.05(0.45-2.45), p_{trend}=0.84

Author (year), country	Total N	Age (y); Mean ± SD; (min-max)	Female (%)	Vitamin D, 25(OH)D unless stated		Cognitive tests; Dementia Criteria	Results
Slinin (2010)(387), USA	1606	≥65	0	LC-MS In-house	Q1: <49.8 Q2: 62.6 Q3: 74.4 Q4: >74.4	3MS Trials B	<u>Prevalent CI: Adjusted OR (95% CI) adjusted cognitive impairment at baseline, Q4 reference</u> *3MS<3 (n=1,598):* Q1: 0.93 (0.36,2.39); Q2: 1.24 (0.49, 3.13);Q3: 0.98 (0.38, 2.52), p_{trend} =0.97 *Trials B<1.5SD(n=1559):* Q1: 1.09 (0.61, 1.93); Q2: 0.81 (0.45, 1.48);Q3: 1.14 (0.65, 2.00), p_{trend} =0.96 <u>Incident CI: Adjusted OR(95%CI) at follow-up, Q4 reference</u> *3MS(n=1138):* Q1: 1.41 (0.89, 2.23); Q2: 1.28 (0.84, 1.95);Q3: 1.06 (0.70, 1.62), p_{trend} =0.10 *Trials B(n=1051):* Q1: 1.08 (0.53, 2.19); Q2: 1.08 (0.57, 2.04);Q3: 0.96 (0.50, 1.82); p_{trend} =0.75,
Slinin (2012)(388), USA	6257	≥65	100	Mass spec- In-house	≤25 50-62.4 62.5-75 ≥75	MMSE TMT B	<u>Baseline: OR (95%CI) with >=30 referent</u> *MMSE:* <10ng/ml(n=425) 1.60(1.05-2.42); 10-19(n=2037)1.09(0.82-1.44); 20-29(n=2408) 0.79(0.60-1.03), p_{trend} = 0.03 *Trials B:* <10ng/ml(n=425) 1.09(0.68-1.76); 10-19(n=2037)1.23(0.90-1.68); 20-29(n=2408) 1.12(0.82-1.52), p_{trend} = 0.31 <u>Follow-up. Decline is >1SD below with mean difference OR (95%CI) with >=30 referent</u> *MMSE:* <10ng/ml(n=425) 1.58(1.12-2.22); 10-19(n=2037)1.31(1.042-1.64); 20-29(n=2408) 1.13(0.91-1.41), p_{trend} = 0.003 *TMT B*: <10ng/ml(n=425) 1.00(0.64-1.56); 10-19(n=2037)0.93(0.70-1.23); 20-29(n=2408) 0.83(0.64-1.09), p_{trend} = 0.93

Author (year), country	Total N	Age (y); Mean ± SD; (min-max)	Female (%)	Vitamin D, 25(OH)D unless stated		Cognitive tests; Dementia Criteria	Results
Llewellyn (2010)(389), Italy	858	74.0±6.8	56.8	RIA DiaSorin	Q1: <25 Q2: <50 Q3: <75 Q4: >75	MMSE TMT A and B	Baseline, score (SD) *MMSE:* Q1(n=175): 23.7(5.3); Q2(n=360): 25.2(3.3); Q3(n=166):26.0(3.0); Q4(n=157): 26.3 (2.8) *p*<0.001; 2-way interaction *p*=0.76 *TMT A:* Q1: 151.8(94.7); Q2: 114.9(75.5); Q3:94.2(69.7); Q4:87.2 (62.1) *p*<0.001; 2-way interaction *p*=0.46 *TMT B*: Q1:239.9(78.4); Q2:219.6(80.8); Q3:118.5(32.4); Q4:180.5(87.0) *p*<0.001; 2-way interaction *p*=0.91 *Dementia n(%):* Q1:16(9.1); Q2:10(2.8); Q3:2(1.2); Q4:1(0.6), *p*<0.001 Adjusted RR (95%CI) 6-year substantial cognitive decline with Q4 as reference *MMSE:* Q3: 1.19 (0.84 to 1.58); Q2: 1.09(0.78 to 1.43); Q1: 1.60 (1.19 to 2.0), p_{trend} =0.02 *Trials A*: Q3: 0.95(0.55 to 1.51) ; Q2: 1.25(0.75 to 1.71); Q1: 1.16(0.65 to 1.84), ptrend=0.44 *Trials B:* Q3: 0.99(0.74 to 1.23) ; Q2: 1.11(0.88 to 1.32); Q1: 1.31(1.03 to 1.51), p_{trend} =0.04 Adjusted Beta(SE) year-on-year change with Q4 as reference *MMSE:* Q3: -0.11(0.12); Q2:-0.035(0.095); Q1:-0.32(0.109), ptrend=0.03 (all participants) *MMSE:* Q3: -0.13(0.114); Q2:-0.051(0.096); Q1:-0.310(0.109), p_{trend} =0.04 (non-demented only)

Author (year), country	Total N	Age (y); Mean ± SD; (min-max)	Female (%)	Vitamin D, 25(OH)D unless stated		Cognitive tests; Dementia Criteria	Results
Breitling (2012)(390), Germany	1639	74.1±2.8 73.9±2.7	60.2	Chemilumine scence DiaSorin (for women) DIS (for men)	Women Q1: 17 Q2: 24 Q3: 32 Q4: 45 Men Q1: 37 Q2: 51 Q3: 25 Q4: 79	COGTEL	Women: fully adjusted beta-coefficient (95% CI) with Q5 as reference: Q4: -0.43(-2.33 to 1.47; Q3: -0.76(-2.69 to 1.13); Q2: -0.08 (-2.06 to 1.90): Q1: -1.91(-3.92,0.09), *p*=0.31 non-linearity *p*=0.015 Men: fully adjusted beta-coefficient (95% CI) with Q5 as reference: Q4: -0.44(-2.52 to 1.64; Q3: 1.64(-0.71 to 3.38); Q2: -0.90 (-3.02 to 1.23): Q1: -1.05(-3.21 to 1.11), *p*=0.15 non-linearity, *p*=0.28
Annweiler (2011)(391), France	40	(76.4-82)	100	RIA Incstar	<25 ≥25	NAD: matching DSM-IV but not matching NINCDS-ADRDA criteria AD: matching NINCDS-ADRDA	Adjusted OR (95% CI), with vitamin D deficiency 19.57 (1.11, 343.69), *p*=0.04 14.95 (1.17, 190.74), stepwise backward model *p*=0.04
Annweiler (2012)(392), France	498	79.8 ±3.8	100	FFQ (dietary intake)	NA	No dementia, AD and Other dementias: DSM-IV and NINCDS-ADRDA	Onset of AD: Adjusted OR (95% CI) 0.99(0.98-0.99), *p*=0.41 Onset of other dementias: Adjusted OR (95% CI) 1.01(1.00-1.02), *p*=0.07
Randomised Controlled Trial							
Rossom (2012)(393), USA	4143	Treatment (65-80) Control (65-80)	Treatment 100 control 100	NA	NA	Probable dementia MCI Global cognitive function	Hazard Ratio (95% CI) of Probable Dementia or MCI for treatment versus control: 0.94(0.72-1.24), *p*=0.68

Author (year), country	Total N	Age (y); Mean ± SD; (min-max)	Female (%)	Vitamin D, 25(OH)D unless stated		Cognitive tests; Dementia Criteria	Results
Manders (2009)(394), Netherlands	176	Treatment median 83 (72.9-92.0 p10-p90) Control 83.0 (70.8-91.4)	Treatment 69.7 Control 68.4	ELISA DiaSorin		ADAS-cog(total score, memory/orientation, language, and Praxis components) MMSE Verbal fluency test (animal and professions)	<u>Difference in cognitive tests in treatment (n=78) vs. controls (n=33)</u> *Animal test:* 0.6(3.4) vs. 0.0(3.2), *p*=0.41 *Profession test:* -0.1 (3.0) vs. -0.3 (2.6), *p*=0.687 *ADAS-cog total:* 0.0 (-5.0, 8.0) vs. 1.0 (-5.6, 6.0), *p*=0.845 *Memory*: 0.0 (-4.8, 5.0) vs. 0.0 (-3.0, 5.0), *p*=0.79 *Language:* 0.0 (-1.0, 2.7) vs. 0.0 (-2.6, 3.0), *p*=0.884 <u>Vitamin D concentration:</u> 21.0 (-2.7, 57.9) vs. -0.5 (-5.4, 24.8), *p*<0.001
Chandra (2001)(395), Canada	96	Treatment 75 Control 74	Treatment 58.3 control 56.3	CPBA Unknown	NA	MMSE Wechsler memory Halstead-Reitan categories Buschke consistent long-term retrieval Digit span (forward) Salthouse listening span Long-term memory recall	<u>For all cognitive tests</u> All participants combined: no significant correlation between levels of individual nutrients and cognitive function test scores, *p*-value not reported; No single nutrient appeared to influence cognition, *p*-value not reported Significant difference between treatment and control groups for all tests

Author (year), country	Total N	Age (y); Mean ± SD; (min-max)	Female (%)	Vitamin D, 25(OH)D unless stated		Cognitive tests; Dementia Criteria	Results
Before-after study							
Annweiler (2012)(396), France	44	Treatment median 81.06, IQR 14.0	Treatment 54.5	NA	NA	MMSE CAB FAB	OR (95% CI) for improvement in cognitive tests: MMSE: 3.80(1.02-14.21), p=0.047 CAB: 16.50(2.51-108.60), p=0.004 FAB: 8.00(1.52-42.04), p=0.01
Przybelski (2008)(397), USA	63	Treatment 86.2 (2.3SE)	Treatment 68.0	LC-MS In-house	NA	CDT Verbal fluency test	Mean total 25(OH)D, ng/ml after 4 weeks: n=25: 17.3 to 63.8, *p*<0.001 Verbal fluency mean (SEM). Baseline vs. treatment 8.3 (1.2) vs. 8.3 (1.2) CDT: 5.1 (0.5) vs. 5.7 (0.5)

1,25OHD (1,25 dihydroxyvitamin D); 3 MS (Modified Mini-mental State Examination); AD (Alzheimer's Disease); ADAS-cog (Cognition part of the Alzheimer's Disease Assessment Scale); AMT(Abbreviated Mental Test Score); APOE (Apolipoprotein E); ATD (Alzheimer's Type Dementia); BMI (Body Mass Index); CAB (Cognitive Assessment Battery); CalCAP (California Computerized Assessment Package); CANTAB (Cambridge Neuropsychological Testing Automated Battery); CDR (Clinical Dementia Rating); CDT (Clock Drawing Test (Wolf-Klein)); CHI (Cognitively Healthy Individuals); CIC (Chromsystems Instruments and Chemicals); COGTEL (Cognitive Telephone Screening Instrument); COWA (Controlled Oral Word Association); CPBA (Protein Binding Assay); CREST (Consortium for Research in Elder Self-neglect of Texas); CRIN (Clinical Research in Neurology); CSI (Clinical Significant Impairment); CSI-D (Community Screening Instrument for Dementia); CTRM (Camden Topographical Recognition Memory); CVLT (California Verbal Learning Test); CWI (Colour Word Trial); DSM-III-R (Diagnostic and Statistical Manual of Mental Disorders, 3rd Edition, Revised); DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, 4th Edition); DSST (Digit-Symbol Substitution Test); EIA (Enzyme Immuno Assay); EPIDOS (Epidemiologie de l'Osteoporose); ELISA (Enzyme-linked Immunosorbent assay); EMAS (European Male Ageing Study); FAB (Frontal Assessment Battery); FFQ (Food Frequency Questionnaire); FST (Faces Symbol Test); GAIT (Gait and Alzheimer Interaction Tracking study); HII (Hachinski Ischaemic index); HPLC (High Performance Liquid Chromatographic system); HSE (Health Survey for England); HVLT-R (Hopkins Verbal Learning Test-Revised); IDS (Immunodiagnostic System Limited); IU (International Units); LC-MS (Liquid Chromatography-tandem Mass Spectrometry); MCI (Mild Cognitive Impairment); MMSE (Mini-Mental State Examination); MRI (Magnetic Resonance Imaging); MrOS (Osteoporotic Fractures in Men); MS (Multiple Sclerosis); MTS (Mental Test Score); NAART (North American Adult Reading Test); NAD (Non-Alzheimer's Dementia); NAME (Nutrition and Memory in Elders); NART (National Adult Reading Test); NHANES (National Health and Nutrition Examination Survey); NINDS-ADRDA (National Institute of Neurological Disorders and Stroke – Alzheimer's Disease and Related Disorders Association criteria); NINDS-AIREN (National Institute of Neurological Disorders and Stroke –Association Internationale pour la Recherch et l'Enseignement en Neurosciences criteria); PASAT (Paced Auditory Serial Addition Task); PHPT (Primary hyperparathyroidism); RIA (RadioImmunoAssay); ROCF (Rey-Osterrieth Complex Figure); RVDLT (Rey Visual Design Learning Test); SBT (Short Blessed Test); SDAT (Senile Dementia of Alzheimer Type); SPMSQ (Pfeiffer Short Portable Mental State Questionnaire); TMT (Trail-making test); VTD (Vascular Type Dementia); WAIS-III (Wechsler Adult Intelligence Scale-Third Edition); WMC-III LM (Wechsler Memory Scale-Third Edition, logical memory); WMS-III (Wechsler Memory Scale-Third Edition); ZENITH (Zinc Effects in Nutrient/Nutrient Interactions and Trends in Health and Ageing)

The review identified some evidence of an association between vitamin D and cognitive function, however results remain equivocal. The majority of studies identified were cross-sectional in design (*n*=26). Of these studies, 73% found a significant association between vitamin D and either cognitive function or dementia criteria, whereby participants with lower 25(OH)D concentrations were more likely to perform worse on cognitive tests or have dementia compared with those with higher concentrations (324, 355, 357, 359, 360, 363-365, 367-372, 374-376, 378, 379).

The second most common study design was case-control (*n*=8). Although mean 25(OH)D concentrations were found to be significantly lower amongst those with dementia in some studies (381, 385, 386), results remain inconclusive with five case-control studies finding no significant difference in 25(OH)D concentrations between cases and controls (333, 380, 382-384).

There were seven cohort studies identified of which five found a significant association between 25(OH)D concentrations and cognitive function or dementia (388-392). One study found a significant association between vitamin D intake and Alzheimer's disease, but not for other dementias (392) whilst another identified an association between 25(OH)D concentrations and performance on the Mini-mental State examination (MMSE) but not for the Trail -making Test B (388). Furthermore, a non-linear relationship between 25(OH)D concentrations and cognitive performance was observed amongst women (390).

Three randomised controlled trials (RCT) examining the effect of vitamin D on cognitive function have been performed. The largest one was conducted amongst a group of women aged ≥65 years with no dementia at baseline (*n*=4,143) (393). There was no difference in developing dementia or mild cognitive impairment (MCI) between the treatment group (receiving 400IU vitamin D and 1,000mg calcium carbonate) compared with the placebo group over the follow-up time of 7.8 years. It is difficult to determine what effect vitamin D supplementation alone may have had on the development of dementia or MCI. Additionally, the dose of vitamin D supplementation may have been too low to have an effect. The second largest RCT was completed

in the Netherlands amongst participants in a nursing home aged >60 years, who had a BMI of ≤30 kg/m^2 and MMSE score of at least 10 points (*n*=176) (394). There was no overall effect of treatment with a nutrient dense drink (containing vitamin D, twice a day) on the Alzheimer's Disease Assessment Scale (ADAS) or a test of verbal fluency. However, there was a beneficial effect of the nutrient dense drink amongst a subgroup of participants with a low BMI (<24.4kg/m^2 at baseline). This RCT was conducted over a period of 24 weeks which may be too short to see neuropsychological improvements. The final RCT was carried out amongst 96 participants aged >65 years (395). The treatment group received a supplement of trace elements (with 4µg vitamin D) over one year. While cognitive function (i.e. immediate and long-term memory, abstract thinking, problem-solving ability and attention) improved, there was no correlation between individual nutrients and cognitive function.

Of the two before-after studies with a comparison group design, one found a significant improvement in cognitive scores with vitamin D3 supplementation over a 16 month period (n=44) (396). The other before-after study found no improvement after 4 weeks supplementation with vitamin D2 amongst 63 nursing home residents (397).

These heterogeneous results may be partly due to study differences. Sample sizes varied from 25 (357) to 11,232 (355) as did the study populations for example, community, prison, hospital, clinic or institutionalised groups. Furthermore, some studies included only men (335, 357, 359, 387) whilst others focused exclusively on women (362, 371, 376, 378, 381, 382, 385, 386, 388, 391-393). Methods of measuring 25(OH)D also varied, with the most frequent one being the DiaSorin RIA method (*n*=18). Additionally various vitamin D cut points were used for example, <25nmol/l, ≥25-50nmol/l, <50nmo/l or quartiles and quintiles. There was also considerable variation in how the outcome was defined. In 20 studies, the outcome included a diagnosis of dementia or cognitive impairment (for example, mild dementia, Alzheimer's dementia, vascular type dementia). Of the studies with a test of cognitive function, the most common was MMSE (*n*=14). Other cognitive domains measured included memory, attention/processing speed, executive function, verbal fluency and intelligence. Another factor which may have influenced the observed estimates

was the control for potential confounders. While some studies used minimal adjustment for example, age, gender, season and ethnicity, others included a range of potential confounders for example, BMI, education, chronic illness, smoking, alcohol, physical activity, family income, other nutrients (vitamin E, zinc B1, B6 and B12) amongst others see **Appendix 4.2** for details.

Interestingly, results from a random effects meta-analysis conducted in older adults (age ≥65 years) consisting of various study designs found significantly lower mean 25(OH)D concentrations amongst those with Alzheimer's disease compared with controls (weighted mean difference: -6.2nmol/l 95% CI -0.6 to -1.8). They also observed that those with <50nmol/l 25(OH)D performed significantly worse on the MMSE when compared with those with ≥50nmol/l (average difference in MMSE score:1.2 95% CI 0.05 to 1.9) (185).

Despite promising results from the meta-analysis, much remains unknown about the relationship between 25(OH)D and cognitive function. Most of the evidence to date has been obtained from studies which have mainly focused on older adult populations (≥65 years). Therefore, the association between 25(OH)D and cognitive function amongst adults in mid-life remains unexplored. The examination of the relationship between 25(OH)D and cognitive function in mid-adulthood could be essential for identifying the timing of successful interventions to maintaining cognitive function.

There are many potential confounders of the relationship between 25(OH)D and cognitive function. While some studies have attempted to adjust for potentially important confounding factors such as education, others have failed to do so (**Appendix 4.2**) (355). Research on influences on cognitive function in adulthood and later life is further complicated by the fact that they need to take account of strong influences in earlier life factors on cognitive development. Cognitive ability in childhood is an important predictor of cognition function in later life and may protect against cognitive decline in mid-life (112). Childhood cognitive ability may also influence health-related behaviours and educational attainment in mid-life which could mediate the effects of cognitive function in later life. Early life factors such as childhood cognitive ability and educational attainment could also influence vitamin D-related behaviours in mid-life such as

sun-exposure behaviour and use of dietary supplements containing vitamin D, which may explain the association between 25(OH)D and cognitive function in mid-life. Therefore, this chapter uses observational epidemiology to examine the prospective association between 25(OH)D and cognitive function in mid-life in the presence of earlier life factors (**Chapter 2**).

6.2 Data and methods

All data used in this chapter, including how 25(OH)D concentrations were collected, administration and standardisation of cognitive tests and the earlier life factors of childhood cognitive ability and educational attainment were described in detail in **Chapter 4**. Here, I will give a brief overview of the relevant data for this chapter.

6.2.1 Participants

Information was obtained from members of the 1958 British birth cohort (1958BC). For the current analyses, information was collected in childhood (7, 11 and 16 years) and adulthood (23, 31, 42, 45, 46 and 50 years). Eligible participants included those who completed at least one cognitive test at 50 years and had 25(OH)D measurements from 45 years, were not pregnant and from a European ancestry (*n*=6,496). Study samples varied from 6,378 for letter cancellation to 6,496 for animal naming and immediate word list. **Figure 6.1** illustrates participant selection for this chapter.

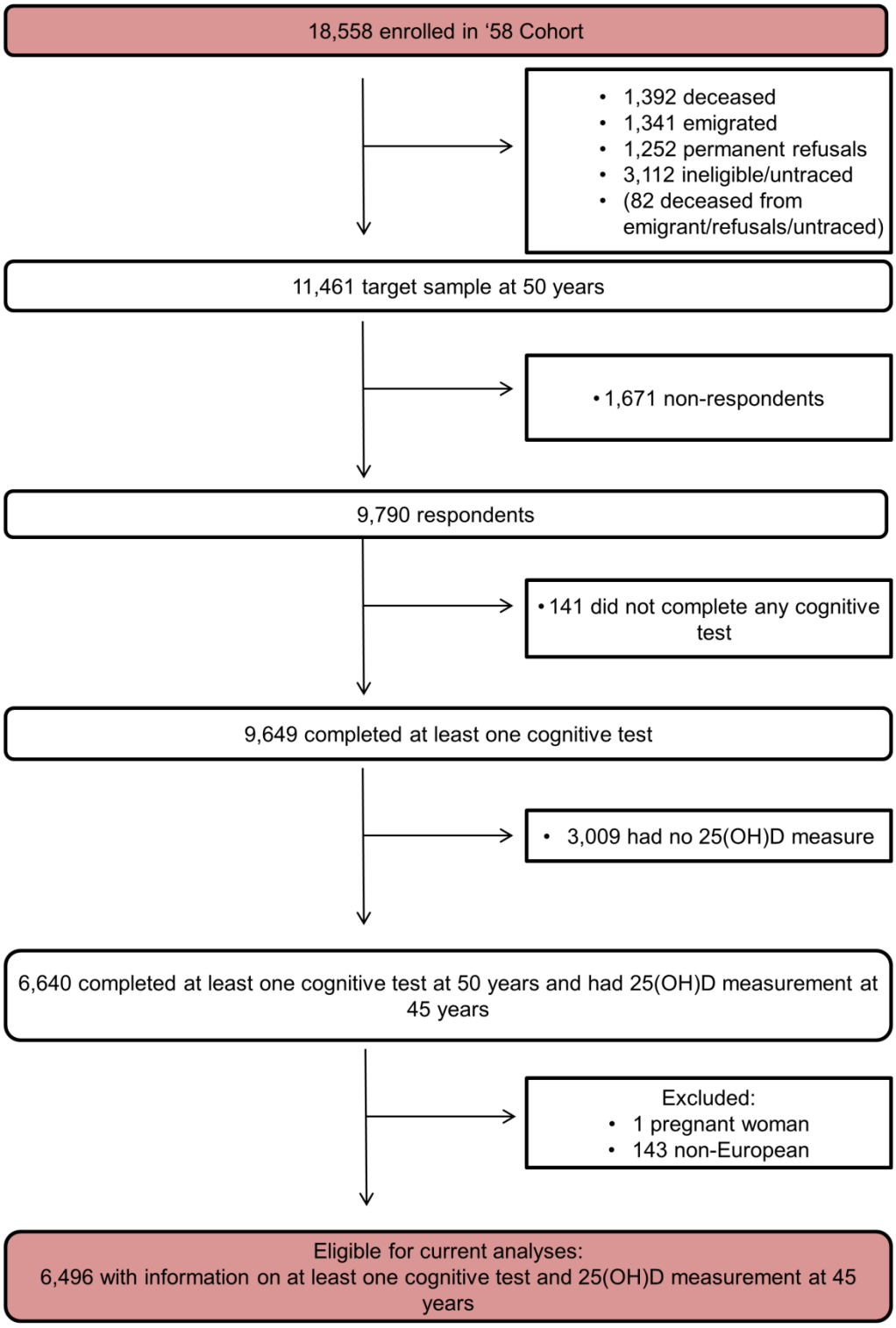


Figure 6.1: Participant selection for study on 25(OH)D and cognitive function

6.2.2 Variables

25(OH)D concentrations were used to assess vitamin D status. Categorisation of 25(OH)D was based on previously established thresholds (**Box 5.1**) (42, 44)

Verbal memory, verbal fluency and speed of processing were the cognitive domains assessed during this chapter (**Chapter 4**). Pearson correlations between these cognitive tests ranged from 0.08 to 0.65; $p<0.001$, for all. Factors that may have influenced performance on cognitive tests were controlled for in the analyses (**Table 4.4**).

A brief description of these earlier life factors and all other covariates used in these analyses are described in **Table 6.2**.

Table 6.2: Covariates from 1958BC for study on 25(OH)D and cognitive function

Covariate	Age of measurement (y)	Description
Educational attainment	Highest qualification obtained by 42 years (or by 33 years if missing)	None or some qualifications, O-level (or equivalent), A-level (or equivalent) or higher degree
Cognitive ability in childhood	7, 11 and 16 years	Tests from each year were standardised for age at assessment and averaged to obtain a summary score for childhood cognitive ability. Missing data on one test were replaced by that individual's mean standardised score on the other tests.
Region of residence	46	Southern England and Channel Islands (South), Middle England and Wales (Middle), Northern England and Isle of Man (North) or Scotland.
SEP* in childhood	Father's occupation at birth (or at 7 years if missing)	Professional and managerial (I and II), non-manual (IIINM), manual (IIIM) and unskilled (IV and V).
SEP in adulthood	42	I and II, IIINM, III, IV and V and unknown.
Smoking	42	Never or former and current
Alcohol	45	Non-drinker, light drinker (<7 units/week), moderate (7-14 units/week), heavy (14-21 units/week) or very heavy (>21 units/week).
BMI (kg/m2)	45	Participants weight (kg) divided by height (m2). Obesity=BMI ≥30kg/m2.
Menopause	45	Post-menopausal, pre-menopausal or peri-menopausal (men coded separately)
Physical activity	42	≤1 time per week, 2-3 times per week or 4-7times per week
Depressive symptoms	45	≥2 symptoms in the past week measured by the Clinical-Interview Schedule Revised.(240)
Frequency of consumption of oily fish (i.e. salmon, trout, mackerel, sardines or fresh tuna)	45	Weekly or less than weekly
Frequency of consumption of margarine	45	Weekly or less than weekly
Supplements of cod liver, fish oil or others containing vitamin D	45	Daily or less than daily
Amount of time spent outside during the past month	45	≥3 or <3 hours per day
Leisure time spent using the TV or PC	45	≥3 or <3 hours per day
Frequency of suncover usage	45	Most of the time or rarely
Blistering after sunburn	45	Often, rarely, sometimes or never
Seeking suntan	45	Often, rarely, sometimes or never

*SEP; socioeconomic position

6.2.3 Statistical analysis

Descriptive statistics

Histograms of 25(OH)D and cognitive tests are given in **Appendices 4.3** and **4.4.** The distribution of 25(OH)D was slightly left skewed, therefore geometric means are reported and natural log transformation (ln) was applied when 25(OH)D was used as an outcome (**Chapter 3**). To assess the distribution of 25(OH)D concentrations by participant demographics, gender-adjusted linear regression models were used with naturally log-transformed 25(OH)D as the outcome (**Chapter 3**).

Association with earlier life factors

Logistic regression models (**Chapter 3**), adjusted for gender and socioeconomic position (SEP) in adulthood and childhood were used to examine the association between earlier life factors (i.e. educational attainment and cognitive ability in childhood) and vitamin D-related lifestyles at 45 years. Linear regression models, adjusted for gender, were then applied to establish the association between earlier life factors and performance on 50 year cognitive tests. Here, standardised cognitive tests were used as the outcome.

Prospective association between 25(OH)D and cognitive performance

The prospective association between 25(OH)D and cognitive performance was examined using linear regression models. Categorised 25(OH)D was the exposure (with <25nmol/l as the reference) and performance on standardised cognitive tests at 50 years was the outcome. These regression models were adjusted for:

1) *Gender and measurement conditions*; season of blood collection and factors that may have influenced cognitive testing (**Table 4.4)**
2) *Social conditions and cognitive factors at earlier life stages*; region, childhood and adult SEP, childhood cognition and educational attainment by 42 years in addition to adjustment 1

3) *Physical status and lifestyles*; obesity, menopausal status, smoking, alcohol, physical activity and depressive symptoms in addition to adjustment 2

The association between 25(OH)D and letter cancellation scores was found to vary by gender ($p_{interaction}$=0.04) therefore stratified analyses were undertaken (**Chapter 3**). Associations for other cognitive tests did not vary by gender.

To examine the possibility of a more complex relationship between 25(OH)D and cognitive function, the presence of a non-linear association was investigated (**Chapter 3**). The non-linear association was explored by including the curvature term of 25(OH)D i.e. ($25(OH)D^2$) in the model.

Additional analysis

In order to examine the effect of using other, nutritional supplements in addition to supplements containing vitamin D, regression models were adjusted for any supplement use (**Chapter 3**).

Of the eligible participants for this analyses, 89.1% (*n*=5,791) also had complete data for all covariates. Missing values ranged from 0.35% (*n*=23) for alcohol to 2.34% (*n*=152) for smoking (**Figure 6.2.**). Multiple imputation was used to correct for missing data on covariates and sample inverse probability weights were used to account for potential selection bias (**Chapter 3**). Imputed results are presented, with complete and weighted results in **Appendix 4.7** to **4.8**. A comparison of complete-case, weighted-case and imputed datasets is given in **Table 6.7**.

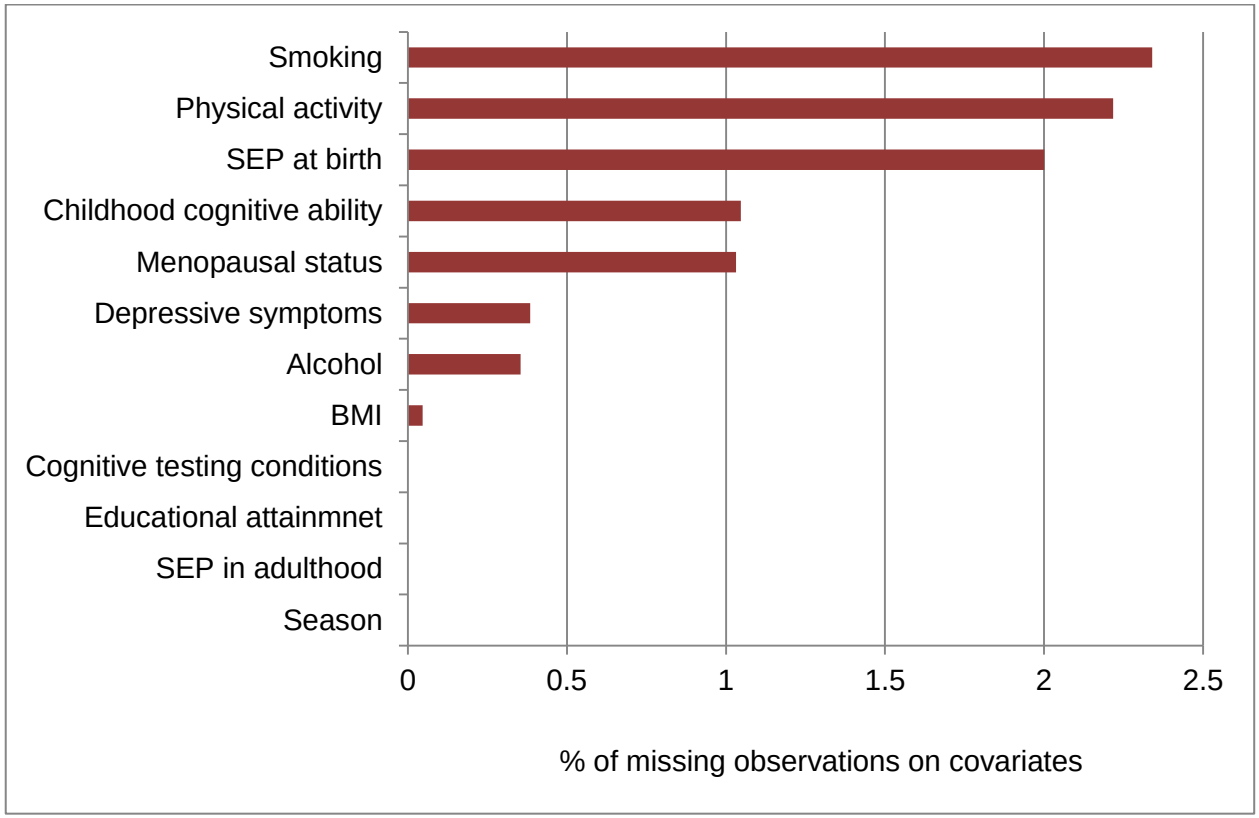


Figure 6.2: Proportion of missing covariate observations for study on 25(OH)D and cognitive function (N=6,496). Missing menopause data relates to women only (n=3,696).

6.3 Results

6.3.1 Descriptive characteristics

Of the 6,496 participants, 49.5% were male. 25(OH)D concentrations ranged from 9.5 to 213.0nmol/l. Geometric mean 25(OH)D concentrations were found to be higher for men (53.6, 95%CI 52.8 to 54.4nmol/l) than women (51.6, 50.7 to 52.4nmol/l, *p*=0.01).

Mean performance on immediate word recall test at 50 years was 6.6 (standard deviation (SD) 1.5), 5.5 (SD 1.8) for delayed word recall test, 22.5 (SD 6.3) for animal naming test and 334.0 (SD 88.1) for letter cancellation test.

25(OH)D concentrations varied according to participant characteristics. For example, those with 50-74.9nmol/l 25(OH)D were more likely to live in southerly regions, be in a higher SEP (i.e. I or II), have a non-obese BMI, be non-smokers, light drinkers and have <2 depressive symptoms (**Table 6.3**). Mean performance on each cognitive test according to 25(OH)D concentrations are displayed in **Table 6.4**.

Performance on cognitive tests varied according to several characteristics (**Appendix 4.5**). Those who performed better on cognitive tests included women, people living in more southerly regions with a higher SEP, non-obese BMI, who exercised 2-3 times per week, were non-smokers, light drinkers and had less than 2 symptoms of depression at 45 years.

Table 6.3: Distribution of 25(OH)D by participant characteristics

	Total	25-Hydroxyvitamin D, nmol/l					P-value*
	N=6,496	<25 *n*=481	25-49.9 *n*=2,177	50-74.9 *n*=2,381	75-99.9 *n*=1,047	≥100 *n*=410	
Season of blood collection (%)							
Winter	1,098	36.4	24.2	12.3	6.9	7.8	<0.001
Spring	1,363	39.1	30.6	16.2	9.4	6.3	
Summer	1,525	12.5	18.6	29.5	26.7	19.3	
Autumn	2,510	12.1	26.7	42.0	57.1	66.6	
Gender (%)							
Male	3,218	40.3	49.8	49.6	52.2	52.2	0.001
Female	3,278	59.7	50.2	50.4	47.9	47.8	
Region (%)							
South	2,503	33.5	38.5	38.9	40.3	37.8	<0.001
Middle	1,674	22.9	25.7	26.7	26.6	22.0	
North	1,692	26.8	24.3	25.8	26.5	34.6	
Scotland	621	16.8	11.4	8.4	6.7	5.4	
Missing	6	0.0	0.1	0.1	0.00	0.2	
SEP in adulthood (%)							
I or II	2,709	39.3	41.9	41.8	41.5	43.4	0.06
IIINM	1,373	21.2	21.4	21.8	20.3	17.8	
IIIM	1,216	15.6	17.6	18.4	22.2	22.0	
IV and V	976	18.5	15.9	15.0	12.3	13.7	
Other/unknown	222	5.4	3.3	3.1	3.7	3.2	
SEP in childhood (%)							
I or II	1,259	14.4	20.3	20.0	19.3	17.1	0.01
IIINM	644	9.2	8.9	10.0	11.8	11.0	
IIIM	3,122	46.6	48.6	47.3	47.5	52.9	
IV and V	1,341	26.0	20.4	21.0	19.0	17.6	
Missing	130	4.0	1.8	1.7	2.5	1.5	
Childhood cognition (mean(sd)) †							
	6,428	0.12 (0.83)	0.14 (0.78)	0.18 (0.77)	0.16 (0.76)	0.08 (0.75)	0.27

	Total	25-Hydroxyvitamin D, nmol/l					P-value*
		<25	25-49.9	50-74.9	75-99.9	≥100	
Educational attainment (%)							
none	504	12.5	8.2	7.2	7.0	5.1	0.13
Some qualifications	898	14.4	13.9	14.0	12.6	15.1	
O level (or equivalent)	1,835	24.5	28.3	27.7	30.2	30.2	
A level (or equivalent)	1,077	16.4	14.8	17.0	18.1	20.2	
Degree	2,182	32.2	34.8	34.1	32.2	29.3	
BMI (kg/m2) (%)							
<30	4,920	63.6	69.4	78.3	83.3	89.5	<0.001
≥30	1,546	35.6	30.0	21.4	16.3	10.0	
Missing	30	0.8	0.6	0.3	0.4	0.5	
Menopause (%)							
Pre-menopausal	2,099	64.5	64.2	63.4	64.7	64.8	<0.001
Peri-menopausal	601	15.0	20.0	19.2	15.8	15.8	
Post-menopausal	123	6.3	3.7	3.3	3.6	4.1	
Other reasons	388	10.8	10.3	12.3	14.0	13.8	
Missing	67	3.5	1.9	1.9	2.0	1.5	
Physical activity (times/week) (%)							
≤1	3,323	59.5	55.5	49.6	44.8	43.4	<0.001
2-3	1,373	16.6	19.0	21.1	26.3	25.1	
4-7	1,656	22.0	23.4	27.2	26.2	29.3	
Missing	144	1.9	2.1	2.1	2.8	2.20	
Smoking (%)							
Never/Former	4,911	59.5	74.0	78.5	78.7	78.8	<0.001
Current	1,433	38.1	23.8	19.4	18.4	18.8	
Missing	152	2.5	2.3	2.1	2.9	2.4	
Alcohol (units/week) (%)							
Non-drinker	371	9.2	7.4	4.9	3.7	2.4	0.002
Light (<7)	3,123	49.5	48.8	48.6	46.1	44.2	
Moderate (7-14)	1,660	19.3	23.2	27.1	28.8	28.5	
Heavy (14-21)	735	8.5	10.6	11.3	13.2	13.7	
Very heavy (>21)	584	12.5	9.7	7.6	8.2	10.7	
Missing	23	1.04	0.3	0.4	0.0	0.5	

	Total	25-Hydroxyvitamin D, nmol/l					P-value*
		<25	25-49.9	50-74.9	75-99.9	≥100	
Depressive symptoms (%)							
<2 symptoms	5,990	86.9	91.8	92.5	93.9	94.6	<0.001
≥2 symptoms	481	12.3	8.0	7.1	5.8	4.9	
Missing	25	0.8	0.2	0.5	0.3	0.5	

25(OH)D, 25-hydroxyvitamin D; SEP, socioeconomic position

* p value for trend is from linear regression adjusted for gender

† Standardised summary score for childhood cognition (age 7-16 years).

Table 6.4: Mean performance on cognitive tests by 25(OH)D status

		Verbal memory				Verbal fluency		Speed of Processing	
		Immediate word recall (Range 0-10)		Delayed word recall (Range 0-10)		Animal naming (Range 0-65)		Letter cancellation (Range 84-780)	
	Total*	Mean	SD	Mean	SD	Mean	SD	Mean	SD
25(OH)D (nmol/l)									
<25	481	6.4	1.6	5.4	1.9	21.5	6.5	335	94
25-49.9	2,177	6.6	1.5	5.5	1.8	22.5	6.3	329	83
50-74.9	2,381	6.7	1.5	5.5	1.8	22.6	6.5	337	90
75-99.9	1,047	6.6	1.4	5.5	1.8	22.5	5.7	335	86
≥100	410	6.4	1.6	5.2	1.8	22.5	6.6	333	91

25(OH)D, 25-hydroxyvitamin D

* N varies according to test; 6,469 for immediate word recall and animal naming, 6,454 for delayed word recall, 6,378 for letter cancellation.

6.3.2 Early life factors

Childhood cognitive ability and educational attainment were associated with vitamin D-related lifestyles in later life (45 years). Participants with higher cognitive scores in childhood (or higher educational attainment) spent less time outside, less time watching TV or using PC in their leisure time, used suncover more often, took more supplements and ate more oily fish than those with lower scores from childhood ($p \leq 0.02$ for all, after adjustment for gender and SEP in childhood and adulthood, **Figure 6.3**). While all behaviours (except time outside, where *more* time outside is associated with higher 25(OH)D concentrations) were associated with a higher mean 25(OH)D in mid-life (**Appendix 3.6**), also see previous work using the 1958BC (26)), there was no association between childhood cognition or educational attainment and 25(OH)D concentrations at 45 years.

Table 6.5 illustrates the association between childhood cognitive ability and educational attainment with cognitive performance at 50 years. Participants with higher cognitive ability and educational attainment performed better on all 50 year cognitive tests (regression coefficients ranged from 0.12 (95% CI 0.09 to 0.15) to 0.45 (95% CI 0.43 to 0.48) and from 0.07 (95% Confidence Interval (CI) 0.05 to 0.09) to 0.22 (95%CI 0.20 to 0.24) respectively ($p<0.001$, for all, adjusted for gender)) (**Appendix 4.6**).

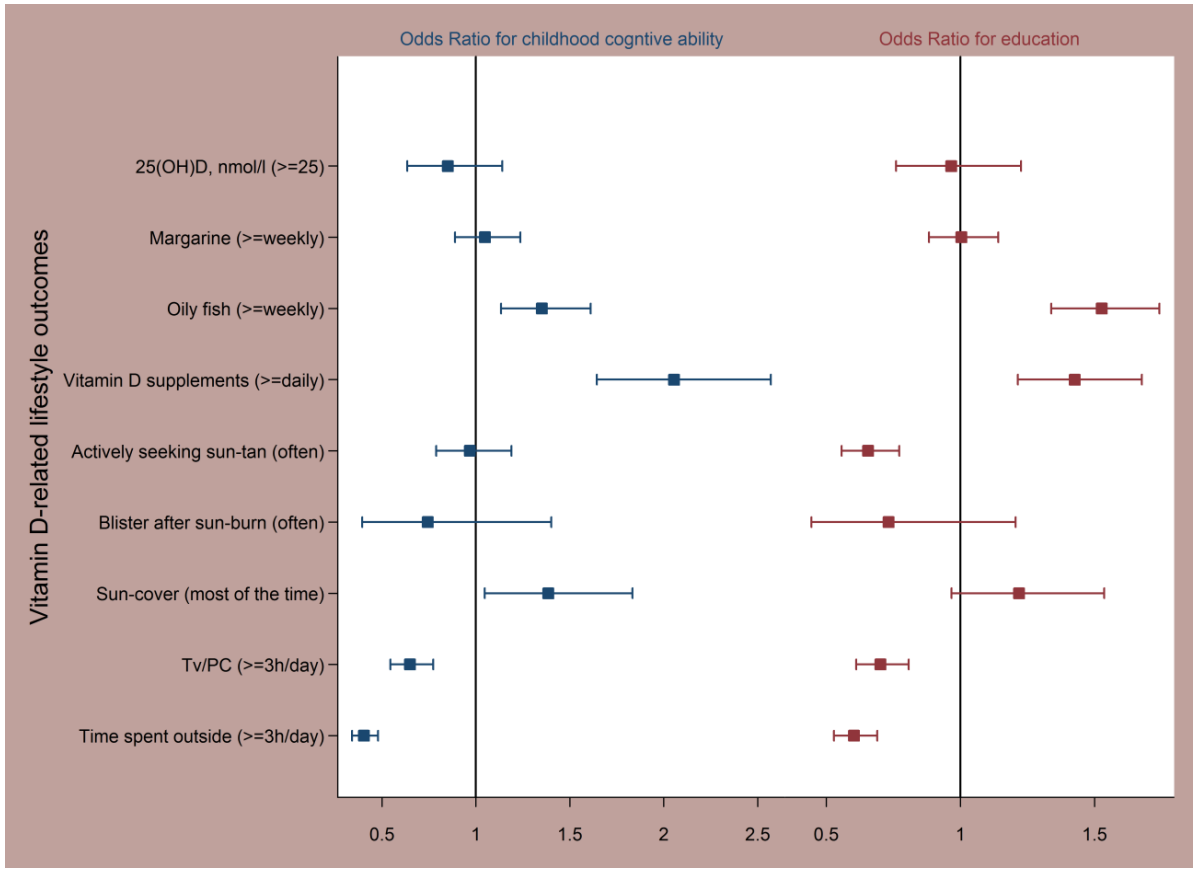


Figure 6.3: Association between childhood cognitive ability and educational attainment with cognitive performance at 50 years

Odds ratio (95%CI) for vitamin D-related lifestyles comparing odds for lowest to highest quartile for childhood cognitive ability (or for no/some/O-level/A-level to degree level education)

Table 6.5: Association between earlier life factors with cognitive performance at 50 years

	Total*	**Immediate word recall** (Range 0-10), mean (SD)	**Delayed word recall** (Range 0-10), mean (SD)	**Animal naming** (Range 0-65), mean (SD)	**Letter cancellation** (range 84-780), mean (SD)
Standardised childhood cognitive function					
<-1 SD	541	5.6 (1.4)	4.3 (1.8)	18.8 (5.6)	319.9 (89.4)
-1 to 0 SD	2,122	6.2 (1.4)	5.1 (1.7)	20.7 (5.6)	327.2 (86.6)
0-1 SD	2,824	6.8 (1.4)	5.8 (1.7)	23.4 (6.1)	337.9 (88.6)
>1 SD	941	7.4 (1.4)	6.4 (1.8)	26.0 (6.6)	345.9 (86.4)
Missing	68	6.4 (1.6)	5.3 (1.8)	21.3 (5.8)	335.8 (99.3)
p †		<0.001	<0.001	<0.001	<0.001
Educational attainment					
None	504	5.8 (1.4)	4.6 (1.8)	19.2 (5.2)	313.5 (86.0)
Some qualifications	898	6.1 (1.4)	4.9 (1.7)	20.1(5.6)	330.3 (84.3)
O-level (or equivalent)	1,835	6.5 (1.4)	5.4 (1.7()	21.9 (5.9)	334.6 (87.63)
A-level (or equivalent)	1,077	6.6 (1.4)	5.5 (1.8)	22.8 (6.6)	323.3 (85.2)
Degree	2,182	7.0 (1.4)	6.0 (1.8)	24.5 (6.4)	345.0 (90.6)
p ‡		<0.001	<0.001	<0.001	<0.001

* N varies according to test: 6,469 for immediate word recall and animal naming, 6,454 for delayed word recall, 6,378 for letter cancellation.

† p value from linear regression adjusted for gender. Missing values are excluded from the analyses

6.3.3 Vitamin D and cognitive performance

Table 6.6 shows the relationship between 25(OH)D at 45 years and performance on cognitive tests at 50 years. There was evidence of a non-linear association between 25(OH)D and immediate word recall ($p_{curvature}$=0.01, for the final model, **Figure 6.4**) with concentrations of both <25nmol/l and ≥75nmol/l 25(OH)D, five years previously, associated with worse performance and levels of approximately 50-75nmol/l associated with better verbal memory scores.

The non-linear relationship between 25(OH)D and cognitive function persisted despite adjustment for childhood cognitive ability and educational attainment and lifestyle factors (i.e. obesity, menopausal status, smoking, alcohol and physical activity and depressive symptoms) (**Figure 6.4**). In fully adjusted models, no associations were observed for 25(OH)D and delayed word recall, animal naming task or letter cancellation score in men and women (**Table 6.6**).

6.3.4 Additional analyses

Weighted and complete case analyses are compared in **Table 6.7 (Chapter 3)**

The additional adjustment of any (non-vitamin D) dietary supplement did not alter the estimates from fully adjusted, complete-case prospective analysis (**Table 6.8)**.

Table 6.6: Association between 25(OH) D at 45 years and cognitive function at 50 years

	25- Hydroxyvitamin D, nmol/l									p_{trend}	$p_{curvature}$
	<25	25-49.9		50-74.9		75-99.9		≥100			
		Coef	95% CI	Coef	95% CI	Coef	95% CI	Coef	95% CI		
Immediate word recall (n= 6,496) *											
Model 1†	1.0	0.12	(0.02 to 0.21)	0.16	(0.06 to 0.26)	0.15	(0.04 to 0.26)	-0.02	(-0.16 to 0.11)	0.76	<0.001
Model 2‡	1.0	0.09	(0.00 to 0.18)	0.12	(0.02 to 0.21)	0.11	(0.00 to 0.21)	-0.03	(-0.15 to 0.10)	0.88	0.001
Model 3§	1.0	0.07	(-0.02 to 0.16)	0.08	(-0.01 to 0.18)	0.06	(-0.04 to 0.17)	-0.07	(-0.20 to 0.06)	0.31	0.01
Delayed word recall (n= 6,454) *											
Model 1†	1.0	0.06	(-0.04 to 0.16)	0.09	(-0.01 to 0.19)	0.06	(-0.05 to 0.17)	-0.08	(-0.21 to 0.05)	0.44	0.01
Model 2‡	1.0	0.04	(-0.05 to 0.13)	0.05	(-0.04 to 0.15)	0.03	(-0.07 to 0.14)	-0.07	(-0.20 to 0.05)	0.34	0.10
Model 3§	1.0	0.03	(-0.06 to 0.13)	0.04	(-0.05 to 0.14)	0.02	(-0.09 to 0.12)	-0.08	(-0.21 to 0.04)	0.22	0.14
Animal naming (n=6,496) *											
Model 1†	1.0	0.14	(0.04 to 0.24)	0.17	(0.06 to 0.27)	0.14	(0.03 to 0.25)	0.16	(0.02 to 0.30)	0.10	0.06
Model 2‡	1.0	0.11	(0.02 to 0.21)	0.11	(0.02 to 0.21)	0.08	(-0.03 to 0.19)	0.13	(-0.00 to 0.25)	0.42	0.34
Model 3§	1.0	0.11	(0.01 to 0.20)	0.11	(0.01 to 0.20)	0.07	(-0.04 to 0.18)	0.12	(-0.01 to 0.25)	0.54	0.36
Letter cancellation (men) (n=3,156) *											
Model 1†	1.0	-0.00	(-0.15 to 0.15)	0.07	(-0.09 to 0.22)	0.10	(-0.06 to 0.27)	0.07	(-0.13 to 0.26)	0.07	0.21
Model 2‡	1.0	-0.03	(-0.17 to 0.12)	0.04	(-0.11 to 0.19)	0.08	(-0.09 to 0.25)	0.06	(-0.13 to 0.26)	0.07	0.35
Model 3§	1.0	-0.04	(-0.18 to 0.11)	0.03	(-0.12 to 0.18)	0.07	(-0.09 to 0.24)	0.06	(-0.13 to 0.23)	0.07	0.49
Letter cancellation (women) (n=3,222) *											
Model 1†	1.0	-0.05	(-0.18 to 0.08)	0.04	(-0.10 to 0.17)	-0.05	(-0.20 to 0.10)	0.01	(-0.19 to 0.20)	0.73	0.23
Model 2‡	1.0	-0.06	(-0.19 to 0.08)	0.03	(-0.10 to 0.17)	-0.05	(-0.20 to 0.11)	-0.00	(-0.19 to 0.19)	0.74	0.26
Model 3§	1.0	-0.07	(-0.20 to 0.07)	0.00	(-0.14 to 0.14)	-0.10	(-0.25 to 0.06)	-0.05	(-0.25 to 0.15)	0.67	0.36

*N represent final model for imputed covariates. Outcomes were not imputed;
†adjusted for gender, season, day and time of cognitive testing, presence of others in the room, other contextual factors affecting performance (i.e. blind or poor eyesight, deaf or hard of hearing, too tired, illness or physical impairment that affects ability to perform, impaired concentration, very nervous or anxious, mental impairment, interruption or distraction, noisy environment, problems with the laptop or difficulty understanding English), word list & method of delivery (latter two for immediate & delayed word recall only);
‡additionally adjusted for region, SEP at 42 years, SEP at birth (7 years if missing), childhood cognition and educational attainment by 42 years;
§additionally adjusted for obese BMI, menopausal status; smoking, alcohol, physical activity; depressive symptoms at 45 years.

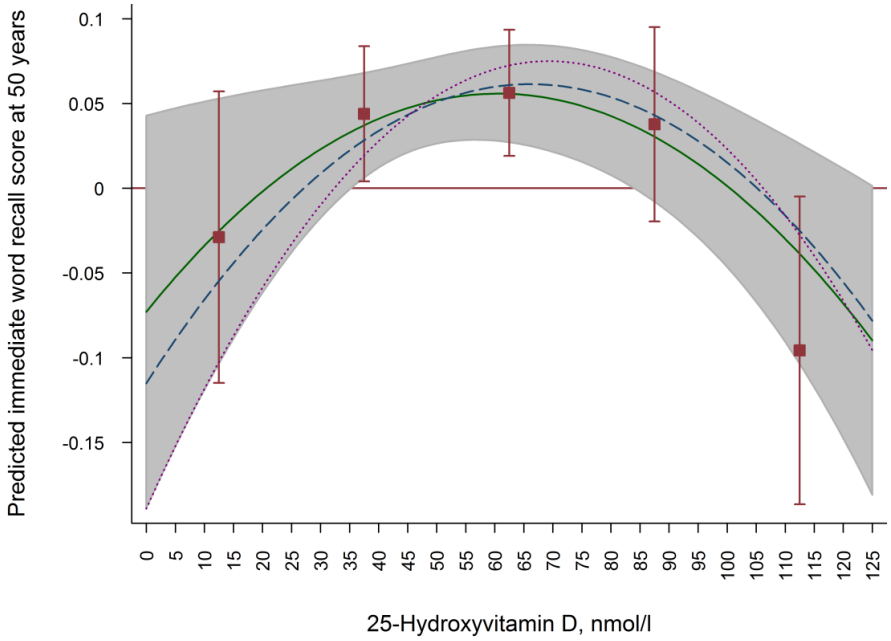


Figure 6.4: Predicted immediate word recall at 50 years according to 25(OH)D concentrations at 45 years

Values are predicted immediate word recall (95% Prediction Interval (PI) at 50 years for fully adjusted models. Dot line adjusted for gender, season, day and time of cognitive testing, presence of others in the room, other contextual factors affecting performance, word list & method of delivery. Dash additionally adjusted for region, SEP at 42 years, SEP at birth (7 years if missing), childhood cognition and educational attainment by 42 years. Solid line additionally adjusted for obese BMI, menopausal status; smoking, alcohol, physical activity; depressive symptoms at 45 years. Shaded areas show 95% PI for fully adjusted models.

Table 6.7: Comparison of complete, weighed and imputed results for 25(OH)D association with immediate word recall

	25- Hydroxyvitamin D, nmol/l									p_{trend}	$p_{curvature}$
	<25	25-49.9		50-74.9		75-99.9		≥100			
		Coef	95% CI	Coef	95% CI	Coef	95% CI	Coef	95% CI		
Immediate word recall (n= 6,496) *											
Complete case-results (n= 6,039)*	1.0	0.08	(-0.01 to 0.18)	0.08	(-0.01 to 0.18)	0.06	(-0.05 to 0.17)	-0.07	(-0.21 to 0.06)	0.19	0.004
Weighted-case results (n=5935)*	1.0	0.08	(-0.03 to 0.19)	0.08	(-0.03 to 0.19)	0.06	(-0.06 to 0.18)	-0.08	(-0.23 to 0.07)	0.20	0.01
Imputed results (n= 6,496)*	1.0	0.07	(-0.02 to 0.16)	0.08	(-0.01 to 0.18)	0.06	(-0.04 to 0.17)	-0.07	(-0.20 to 0.06)	0.31	0.01

Coef, Beta-coefficient from linear regression model

*adjusted for gender, season, day and time of cognitive testing, presence of others in the room, other contextual factors affecting performance (i.e. blind or poor eyesight, deaf or hard of hearing, too tired, illness or physical impairment that affects ability to perform, impaired concentration, very nervous or anxious, mental impairment, interruption or distraction, noisy environment, problems with the laptop or difficulty understanding English), word list & method of delivery (latter two for immediate & delayed word recall only); region, SEP at 42 years, SEP at birth (7 years if missing), childhood cognition and educational attainment by 42 years, obese BMI, menopausal status; smoking, alcohol, physical activity; depressive symptoms at 45 years.

Table 6.8: Association between 25(OH) D at 45 years and cognitive function at 50 years, adjusted for dietary supplements

	25- Hydroxyvitamin D, nmol/l									p_{trend}	$p_{curvature}$
	<25	25-49.9		50-74.9		75-99.9		≥100			
		Coef	95% CI	Coef	95% CI	Coef	95% CI	Coef	95% CI		
Immediate word recall *											
(n=6,039)†	1.0	0.08	(-0.01 to 0.18)	0.08	(-0.01 to 0.18)	0.06	(-0.05 to 0.17)	-0.07	(-0.21 to 0.06)	0.19	0.004
(n= 5,968)‡	1.0	0.09	(-0.01 to 0.19)	0.08	(-0.02 to 0.18)	0.06	(-0.06 to 0.17)	-0.08	(-0.21 to 0.06)	0.12	0.01
Delayed word recall *											
(n=6,000) †	1.0	0.05	(-0.05 to 0.14)	0.05	(-0.05 to 0.15)	0.02	(-0.09 to 0.13)	-0.08	(-0.21 to 0.05)	0.16	0.08
(n=5,929) ‡	1.0	0.05	(-0.05 to 0.15)	0.04	(-0.06 to 0.14)	0.001	(-0.11 to 0.11)	-0.09	(-0.23 to 0.04)	0.07	0.14
Animal naming *											
(n=6,039)†	1.0	0.10	(0.00 to 0.20)	0.10	(-0.01 to 0.20)	0.05	(-0.06 to 0.17)	0.09	(-0.04 to 0.23)	0.90	0.37
(n=5968)‡	1.0	0.10	(0.01 to 0.21)	0.10	(-0.003 to 0.21)	0.05	(-0.06 to 0.17)	0.09	(-0.05 to 0.23)	0.98	0.38
Letter cancellation (men) *											
Model 3 (n=2,955)†	1.0	-0.03	(-0.19 to 0.12)	0.03	(-0.13 to 0.19)	0.06	(-0.12 to 0.24)	0.08	(-0.13 to 0.28)	0.09	0.48
Model 2 (n=2901)‡	1.0	-0.04	(-0.20 to 0.12)	0.02	(-0.14 to 0.18)	0.05	(-0.13 to 0.23)	0.07	(-0.14 to 0.28)	0.12	0.52
Letter cancellation (women) *											
n=2,974)†	1.0	-0.06	(-0.20 to 0.08)	-0.00	(-0.15 to 0.15)	-0.13	(-0.30 to 0.03)	-0.07	(-0.28 to 0.13)	0.33	0.36
(n=3,127)‡	1.0	-0.06	(-0.21 to 0.08)	-0.02	(-0.16 to 0.13)	-0.14	(-0.31 to 0.03)	-0.08	(-0.29 to 0.12)	0.27	0.43

*Results based on complete case-analysis

†adjusted for gender, season, day and time of cognitive testing, presence of others in the room, other contextual factors affecting performance (i.e. blind or poor eyesight, deaf or hard of hearing, too tired, illness or physical impairment that affects ability to perform, impaired concentration, very nervous or anxious, mental impairment, interruption or distraction, noisy environment, problems with the laptop or difficulty understanding English), word list & method of delivery (latter two for immediate & delayed word recall only), region, SEP at 42 years, SEP at birth (7 years if missing), childhood cognition and educational attainment by 42 years, obese BMI, menopausal status; smoking, alcohol, physical activity; depressive symptoms at 45 years;

‡additionally adjusted for intake of any dietary supplement (yes/no)

6.4 Discussion

A paper related to findings from this study has been published in the peer-reviewed journal, *British Journal of Nutrition* (398) (**Appendix 1.2**).

Results indicate that childhood cognitive performance and educational attainment are associated with vitamin D-related behaviours at 45 years and performance on cognitive tests at 50 years. A non-linear relationship between 25(OH)D and one measure of verbal memory (i.e. immediate word recall) was observed and remained significant following adjustment for earlier life factors. 25(OH)D concentrations, in the range of 50-75nmol/l were associated with better performance on this verbal memory measure, compared with both lower 25(OH)D (<25nmol/l) and higher 25(OH)D (≥75nmol/l) concentrations. No significant trends were found for other cognitive tests. Only one measure of 25(OH)D was available and it is possible that participants changed behaviour (and consequently their vitamin D status) during the 5 year interval. Therefore, inferences about the association between chronic or concurrent hypovitaminosis D and cognitive performance should be made with caution. Additionally, the influence of childhood cognitive ability on the subsequent association between 25(OH)D and cognitive function may have been underestimated due to differing measures of cognition in childhood and adulthood.

Results from this study were supportive of findings from the meta-analysis cited in the introduction which demonstrated a 1.2 (95% CI 0.5 to 1.9) improvement in scores on the MMSE for participants with ≥50nmol/l compared with <50nmol/l 25(OH)D (185). However, compared with the meta-analysis, results for the 1958BC differ in terms of the age group examined (i.e. 50 years versus ≥65 years), study design (i.e. prospective design versus mixed designs) and cognitive assessment used (i.e. tests of three cognitive domains versus the MMSE, a test of global cognitive function (399)).

Unlike this study, most investigations of the association between 25(OH)D and cognitive function have focused on older adult populations. To my knowledge, only one previous study has investigated a younger adult population (355). This

cross-sectional study examined three age groups; adolescents (12-17 years), younger adults (20-60 years) and older adults (60-90 years). While no association between low 25(OH)D and impaired performance on psychometric measures assessing memory and speed of processing was observed for younger adults, a significant relationship between low 25(OH)D and reduced performance in memory tests was demonstrated amongst older adults. The younger adult category encompassed a wide age range, which reduces comparability to the present study, in which cognitive function was assessed at a specific age (50 years).

While cognitive decline is more evident in later life (105), there is some suggestion that decline can occur in mid-life (112). Detecting an association between 25(OH)D and cognitive function in relatively young adult populations is further complicated by the trajectory of cognitive ageing and neurodegenerative diseases. Many cognitive disorders have a long pre-clinical phase during which the pathology accumulates (123). Therefore, it is difficult to ascertain if associations between 25(OH)D concentrations and cognitive function in mid-life are reflective of pre-clinical dementia or subtle changes in cognitive ability.

Some previous studies (**Table 6.1**) focused on presence of cognitive impairment or dementia while others, including our study, measured performance on individual cognitive tests. Many studies using cognitive impairment or dementia as an outcome were suggestive of an association between higher 25(OH)D concentrations and reduced risk of impairment or dementia (364, 365, 368, 371, 375, 376, 379, 385, 386, 391), however this was not always replicated (335, 362, 377, 380). Evidence for an association between 25(OH)D concentrations and individual cognitive tests were also inconclusive with some (324, 357, 359, 360, 367, 370, 389, 390, 394) but not all (333, 382, 397) producing significant findings.

As discussed in **Chapter 1**, several studies investigating the impact of risk factors on cognitive decline and dementia over the life-course have implied that certain mid-life factors may be particularly relevant for later cognitive functioning (400). Therefore, investigations such as this one that examine the role of 25(OH)D in cognitive function at a time point where age-related decline is just

becoming apparent, are essential for informing the timing and nature of potential interventions (104).

Interpreting the association between 25(OH)D and cognitive function is further complicated by findings of domain-specific associations. In this study, despite the strong correlation (*r*=0.7) between the two measures of verbal memory, no association between 25(OH)D and delayed recall was detected. Additionally, no association was found between 25(OH)D and other cognitive domains examined i.e. verbal fluency and speed of processing. In contrast with our findings, a previous study conducted in an older adult population (65-99 years) in the US, found that 25(OH)D was associated with non-memory domains i.e. executive function (367). Similarly in the US, a study of women aged ≥65 years found a significant association between 25(OH)D and MMSE scores but not for the Trail-Making test B (TMT B), which measures executive function.

The finding of an association between 25(OH)D and performance on one verbal memory test but not the other tests of cognitive domains could be interpreted in two ways. Firstly, vitamin D may have a specialised role in specific brain functions. Further experimental studies are required to give insight into these potential processes. Secondly, the association between 25(OH)D and the other cognitive domains may vary by life-stage. Perhaps the importance of a person's 25(OH)D status in terms of their cognitive function only manifests at later life stages.

An interesting find from this study was the non-linear association between 25(OH)D and cognitive function. Two previous studies have supported this non-linear relationship between 25(OH)D and cognitive function. One study of men aged 40-79 years suggested that reduction in cognitive function was most evident at <35nmo/l 25(OH)D (359). The other study, among adults aged ≥65 years, proposed that improvement in cognitive function plateaus with higher 25(OH)D concentrations (390).

There are many potential explanations for the non-linear prospective association between 25(OH)D and verbal memory two of which have been described in the discussion in **Chapter 5**. Briefly, the non-linear relationship

may be due to a threshold effect whereby higher 25(OH)D concentrations do not provide any additional benefit to the improvement of cognitive function, and may result in a reduction in cognitive performance. Additionally, the non-linear relationship may have an underlying mechanistic explanation whereby very high 25(OH)D concentrations will ultimately lead to a reduction in the biologically active, 1,25$(OH)_2$D. Furthermore, the association between 25(OH)D and verbal memory may be influenced by the potential interactive effect of genetic variants and 25(OH)D on cognitive function, which will be examined in **Chapter 7**.

In contrast to previous studies, our work aimed to incorporate a lifespan approach. Childhood cognitive ability has been found to be strongly related to cognitive function in later life (401). Cognitive ability in childhood has also been associated with reduced mortality (402) which may be mediated by healthy lifestyle choices (403). The importance of educational attainment on cognitive function in adulthood (404) was discussed in **Chapter 1**. The presented results replicate the finding of the importance of childhood cognitive ability and educational attainment on later cognitive function and interestingly, on vitamin D-related lifestyles in mid-life. While this association has not been taken into account in previous studies, our study indicates that higher childhood cognitive ability and educational attainment are linked with behaviours that are associated with 25(OH)D concentrations in later life. Although childhood ability and educational attainment did explain a proportion of the association between 25(OH)D and cognitive function in mid-life, the non-linear relationship persisted despite controlling for these factors. Therefore, it is plausible that 25(OH)D status may independently predict verbal memory in mid-life.

There are a number of potential confounders that may affect the association between 25(OH)D and cognitive function including early-life factors such as low birth weight, and educational attainment (**Chapter 1** for further details). While this study has included some of these potential confounders in analyses, it is difficult to identify and accurately measure all potential confounding using an observational epidemiologic approach (**Chapter 3)**. Furthermore, the association between 25(OH)D and verbal memory may be influenced by the potential interactive effect of genetic variants and 25(OH)D on cognitive function. If this gene-25(OH)D interaction is present, the effect of 25(OH)D on

cognitive test performance will depend on the individual's particular genetic variant. An example of a gene-25(OH)D interaction effect will be explored further in **Chapter 7**.

6.4.1 Conclusion

Findings from this mid-adulthood population suggest that both low and high 25(OH)D concentrations are associated with poorer verbal memory as indicated by immediate word recall in mid-life, albeit with small effect sizes. Childhood cognition and educational attainment appear to contribute to this association, but do not fully explain it.

6.5 Summary

- There is evidence of a link between hypovitaminosis D and poor cognitive function, particularly in older adults, but studies lack a lifespan approach, and effects of reverse causality remain unknown
- The aim of this chapter was to assess the relationship between 25(OH)D and subsequent cognitive performance in mid-adulthood and the influence of earlier life factors including childhood cognitive ability, on this association
- Participants with both low (<25nmol/l) and high (≥75nmol/l) 25(OH)D concentrations at 45 years performed significantly worse on immediate word recall. Associations attenuated after adjustment for childhood cognitive ability, education, and socioeconomic position, however, for immediate word recall test, there was a non-linear association with 25(OH)D even after further adjustment for obesity, menopausal status, smoking, alcohol, physical activity and depressive symptoms at 45 years ($p_{curvature}$=0.01)
- This observational study demonstrates that 25(OH)D concentrations were non-linearly associated with immediate word recall in mid-life. The limitations of observational approaches imply that causality and the effect of unmeasured confounders on the association between 25(OH)D and cognitive function requires further investigation

Chapter 7 Genetic Study: Gene-environment interaction

7.1 Introduction

The aetiology of a particular phenotype can involve discrete actions of environmental and genetic causes, but also interactions between the two. Results from **Chapter 6** identified a significant non-linear relationship between 25-hydroxyvitamin D (25(OH)D) and a measure of memory (i.e. immediate word recall test) amongst 50 year old participants of the 1958 British birth cohort (1958BC). Examining the effect of a gene-environment interaction (GxE) on the relationship between 25(OH)D concentrations and memory function may provide some insight into potentially distinct relationships according to the presence of a genetic variant. The concept of GxE has been described in more detail in **Chapter 3**.

Apolipoprotein E

Apolipoprotein E (apoE) is one of a group of apolipoproteins that are present in the body. The apoE protein is an important component of plasma and brain lipoproteins (405) and is involved in the metabolism of certain lipoproteins (for example, triglycerides and cholesterol). ApoE may also contribute to the process of neuronal repair (406, 407) and play a role in immunoregulation and nerve regeneration (408).

There are three major protein isoforms of human apoE (i.e. E-2, E-3 and E-4) which are determined by the alleles ε2, ε3 and ε4 (409) (**Chapter 4**). There are six possible *APOE* genotypes: ε2/ε2, ε2/ε3 ε2/ε4 ε3/ε3 ε3/ε4 and ε4/ε4 (**Table 7.1**).

There is some evidence to suggest that the ε2 allele of *APOE* may be positively associated with survival amongst older adults while the ε4 allele might be related to reduced longevity, however the frequency of the ε2 allele is too rare in the population to provide a definitive conclusion (410, 411). *APOE* ε4 genotype has been associated with many adverse health conditions including cardiovascular disease and neurodegenerative disorders (412) as well as an overall increase in morbidity and mortality in older adults (413, 414). However,

APOE ε4 has been shown to have a role in protecting against some infectious diseases (415). This suggests that *APOE* ε4 may have provided some evolutionary advantages in terms of pathogen resistance, but it is not advantageous in terms of chronic diseases.

APOE and cognitive function

The ε4 allele of *APOE* is an important genetic risk factor for late-onset Alzheimer's disease (AD) (i.e. onset >65 years) (146, 147, 416) with some studies suggesting that *APOE* ε4 may account for approximately 20-50% of the attributable total risk for dementia (417, 418). The presence of *APOE* ε4 alleles in the human brain has been shown to influence membrane repair, cholesterol transport and other cellular activities that are related to the development of AD (419). Furthermore, a meta-analysis conducted in 1997 found that heterozygous ε4 carriers are at between three and four times higher risk and homozygous ε4 carriers are between ten and twelve times higher risk of developing AD (420).

While *APOE* ε4 is a known risk factor for AD, it may also affect cognitive function in normal ageing . A meta-analysis of 38 studies conducted in 2004 found that *APOE* ε4 carriers performed slightly worse on cognitive tests of global cognitive function, episodic memory and executive functions compared with non-ε4 carriers (145). The authors of this meta-analysis noted that *APOE* ε4 heterozygosity and older age were associated with smaller ε4-related differences in cognitive function between ε4 carriers and ε4 non-carriers (145).

APOE and 25(OH)D

The hypothesised association between 25(OH)D and *APOE* ε4 developed from the realisation of the non-random distribution of *APOE*. In Europe, Africa and North America, *APOE* ε3 alleles are inversely correlated with ε4 (421). In European populations, a strong north-south gradient was recognised whereby higher frequencies of *APOE* ε4 carriers were observed at greater latitudes (422, 423). It is thought that this north-south distributional pattern is due to an evolutionary adaption to environmental factors (422, 423). This adaptation may have provided a survival advantage to ε4 carriers in the presence of reduced

sunlight (422), which could indicate better protection against vitamin D deficiency amongst ε4 carriers (423). Interestingly, *APOE* ε4 tends to be more prevalent in people with darker skin or those living in regions with reduced sunlight compared with those of moderate pigmentation but exposed to high UV concentrations (40).

One study demonstrated significantly higher 25(OH)D concentrations in *APOE* ε4 targeted replacement mice compared with non-ε4 carriers (41). The authors of this study also noted a positive association between *APOE* ε4 and 25(OH)D concentrations in a general human population sample (41). During their commentary, Huebbe et al (41) implied that their finding of an association between *APOE* ε4 and higher 25(OH)D concentrations may be contradictory because both *APOE* ε4 and vitamin D deficiency are associated with a higher risk of chronic illness. The authors hypothesised that this contradictory finding may imply that the relationship between 25(OH)D and adverse health conditions could be modified by presence of *APOE* ε4 genotype.

Due to the potential associations between (1) 25(OH)D and cognitive function (2) *APOE* ε4 and lower cognitive function and (3) *APOE* ε4 and higher 25(OH)D, it is plausible that the association between 25(OH)D and cognitive function may be modified by the presence of *APOE* ε4 alleles. Therefore, this chapter will explore a GxE interaction between *APOE* genotype and 25(OH)D on memory function (**Chapter 2**).

7.2 Data and methods

Data for this chapter come primarily from participants of the 1958BC. To explore if these results replicate in another cohort, data from participants of the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) were also used.

7.2.1 Participants

1958BC

Details of the 1958BC were given in **Chapter 4**. For the current analyses, eligible participants consisted of the subsample of individuals from European ancestry with information on *APOE* genotypes and for whom memory-related cognitive tests were conducted (*n*=4,848) (**Figure 7.1**). Further analyses requiring 25(OH)D concentrations were carried out among 4,644 participants as 204 individuals (4.2% of eligible sample) did not have information on 25(OH)D concentrations. The sample with and without 25(OH)D measurements did not differ in relation to participant characteristics (including gender, region of residence, educational attainment, depressive symptoms, number of *APOE* ε4 alleles and performance on memory tests) ($p \geq 0.12$). Individuals with *APOE* ε2 alleles (*n*=764) were not included in analyses requiring *APOE* ε4 due to the supposed counteractive effects of ε2 and ε4 (40). Participants with *APOE* ε2 alleles tended to have lower educational attainment compared with the rest of the sample ($p=0.04$), however they did not differ in relation to gender, region or depressive symptoms ($p \geq 54$).

PIVUS

Details of PIVUS were given in **Chapter 4**. 1,016 participants were included in the baseline study (70 years) during which blood samples were obtained for DNA extraction and 25(OH)D concentrations. *APOE* genotypes were obtained for 941 individuals. 938 participants with information on *APOE* genotypes also had data on 25(OH)D concentrations and 739 completed memory-related cognitive tests at 50 years. Eligible participants for this study consisted of the subsample of individuals from European ancestry with information on *APOE* genotypes and for whom memory-related cognitive tests were conducted (*n*=739). There were 3 participants (0.41% of eligible sample) with no information on 25(OH)D concentrations. Individuals with *APOE* ε2 alleles (*n*=108) were not included in analyses requiring *APOE* ε4. Participants with *APOE* ε2 alleles not differ in relation to gender, educational attainment or depressive symptoms ($p \geq 13$).

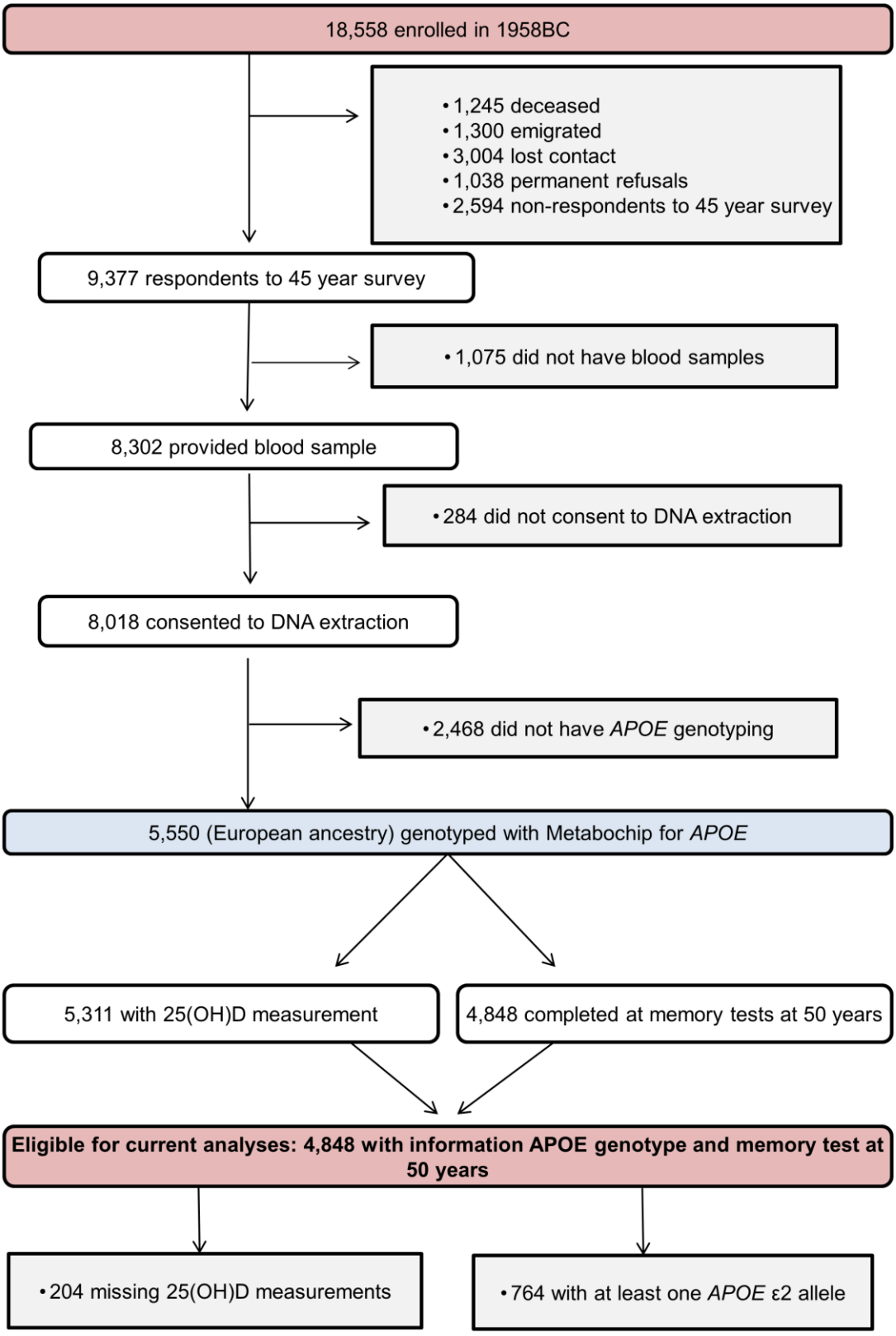


Figure 7.1 Participant selection for GxE study in 1958BC

7.2.2 Variables

APOE

A description of genotyping techniques used to obtain *APOE* in both cohorts is described in **Chapter 4**. Quality checks of genetic variants for *APOE* were conducted following data exclusions. The call rate, minor allele frequency and Hardy Weinberg equilibrium for the SNPs in each study is reported in **Appendix 5.1**. The minor allele frequencies were compared with HapMap data on populations with a European ancestry[6] and were found to be similar.

Frequencies of the six possible *APOE* genotypes obtained from the three main *APOE* alleles i.e. ε2 (rs7412:T and rs429358:T), ε3 (rs7412:C and rs429358:T), ε4 (rs7412:C and rs429358:C) are given in **Table 7.1**.

Since the emphasis for this study was on the ε4 allele, further coding was required. When used as a genotype count model, the number of ε4 alleles was coded as 0-2 depending on the presence of ε4 alleles. This variable did not include any ε2 carriers since the mechanisms for *APOE* ε4 and ε2 on cognitive function may be different (***section 7.1***). Therefore, the reference category was two ε3 alleles. When used in a recessive model, *APOE* ε4 was coded as two ε3 or one ε4 alleles versus two ε4 alleles.

25-Hydroxyvitamin D

Details of how 25(OH)D was measured in both cohorts can be found in **Chapter 4**. Distributions of 25(OH)D in both cohorts can be seen in **Appendix 5.2**. Since 25(OH)D was found to be skewed, geometric means are reported and natural log (ln) transformation was applied to approximate a normal distribution when 25(OH)D was used as a continuous outcome.

[6] http://hapmap.ncbi.nlm.nih.gov/cgi-perl/gbrowse/hapmap27_B36/

Table 7.1: *APOE* genotype frequency

rs7412	**rs429358**	***Allele* 1**	***Allele* 2**	***APOE* Genotype**	**Frequency in 1958BC (%)***	**Frequency in PIVUS (%)†**
TT	TT	ε2	ε2	ε2/ε2	0.60	0.54
	TC	-	-	-	-	-
	CC	-	-	-	-	-
CT	TT	ε2	ε3	ε2/ε3	12.46	11.77
	TC	ε2	ε4	ε2/ε4	2.70	2.30
	CC	-	-	-	-	-
CC	TT	ε3	ε3	ε3/ε3	57.49	58.86
	TC	ε3	ε4	ε3/ε4	23.89	24.09
	CC	ε4	ε4	ε4/ε4	2.87	2.44

*n=4,848; † n=739

Memory function

Evidence from the 1958BC indicated the relationship between 25(OH)D and cognitive function exists particularly in the memory domain (i.e. from a test of immediate word recall) **(Chapter 6)**, therefore this study will focus on memory function to avoid undue multiple testing. Details of how each of these memory-related cognitive tests was conducted in the two cohorts are described in detail in **Chapter 4,** and are displayed in **Appendix 5.3**. Each of these tests were standardised and combined into an overall memory function score as described in **Chapter 4.**

Covariates

When analyses was conducted using participants from 1958BC, the main covariates included gender, month of blood collection, region of residence (categorised as Southern England and Channel Islands (South), Middle England and Wales (Middle), Northern England and Isle of Man (North) or Scotland), education (categorised as none, <O-level or equivalent, O-level or equivalent, A-level or equivalent, degree) and depressive symptoms at 45 years (categorised as yes or no) as measured by the Clinical Revised Interview Schedule-Revised (239, 240). The proportion of missing covariates ranged from 0.02% for region of residence to 12% for education (**Table 7.2**).

Analyses conducted using data from PIVUS used the available covariates of gender, month of blood collection, education (categorised as primary school (<9 years), secondary school (9-2 years), university level (>12 years). Participants of PIVUS did not have information regarding region of residence or depressive symptoms and 1% did not have information on education (**Table 7.2**).

7.2.3 Statistical methods

Details of statistical methods are given in **Chapter 3**. Linear regression models were used to examine associations between 25(OH)D and *APOE* ε4 genotype with memory function. Models were adjusted for gender, month of blood collection, region, education and depressive symptoms, where appropriate.

Firstly, in a similar manner to **Chapter 6**, the association between 25(OH)D and memory function was examined. In addition to standard adjustments, models were also adjusted for *APOE* ε4 genotype to account for the potential effect of *APOE* ε4 alleles on the relationship between 25(OH)D and memory function. Curvature was examined by including the quadratic term of 25(OH)D in the model (**Chapter 3**).

Secondly, the association between *APOE* ε4 genotype and 25(OH)D was examined using a genotype count model. In addition to standard adjustments, models were further adjusted for memory function.

Thirdly, the association between *APOE* ε4 genotype and memory function was examined using a genotype count model. Further adjustments for 25(OH)D concentrations were examined in the model.

Given the presence of a non-linear association between 25(OH)D and memory function identified using observational data from 1958BC (**Chapter 6**), examination of effect modification by *APOE* ε4 genotype was started from the most complex model (**Chapter 3**). For consistency, the interaction models used in the 1958BC were repeated in PIVUS. Stratified analyses and graphical presentation were used to interpret the final interaction model.

Sensitivity analyses was conducted by restricting the sample to those will full information on 25(OH)D and who did not have an *APOE* ε2 allele (1958BC *n*=3,914; PIVUS *n*=629).

7.3 Results

7.3.1 Descriptive characteristics

There were 4,848 and 739 eligible participants from 1958BC and PIVUS respectively. **Table 7.2** illustrates the main characteristics of participants from 1958BC and PIVUS. Gender distribution in both cohorts was approximately equal. The mean age of participants of the 1958BC was 20 years younger than those from the Swedish cohort, PIVUS.

Geometric means of 25(OH)D were slightly higher amongst participants of PIVUS. **Figure 7.2** shows the distribution of participants according to thresholds for 25(OH)D deficiency or insufficiency (i.e. <25 or <50nmol/l) (42, 44). Men were more likely to have higher mean 25(OH)D concentrations than women in both cohorts (p<0.01). In the eligible sample, the proportion of individuals with both <25 and <50nmol/l were higher in 1958BC compared with PIVUS. 25(OH)D concentrations ranged from 9.5 to 169.6nmol/l amongst eligible participants from 1958BC and from 10.9 to 133nmol/l amongst participants from PIVUS.

Table 7.2: Characteristics of eligible participants from 1958BC and PIVUS

	1958BC	PIVUS
Gender, %(N)		
Male	53.9 (2,614)	49.7 (367)
Female	46.1 (2,234)	50.3 (372)
Age at time of cognitive testing, mean (min-max)		
	50 (50-50)	70.2 (69.8-72.2)
25-hydroxyvitamin D, nmol/l geometric mean (min-max)		
	52.4 (9.5-169.6)	55.0 (10.9-133)
missing, % (N)	4.2 (204)	0.4 (3)
Month of 25(OH)D collection, %(N)		
January	7.7 (377)	8.4 (61)
February	7.2 (350)	9.1 (64)
March	7.1 (343)	12.5 (84)
April	6.0 (293)	9.5 (67)
May	6.8 (327)	10.2 (76)
June	9.5 (459)	8.5 (64)
July	7.4 (357)	0 (0)
August	5.6 (269)	6.7 (53)
September	12.1 (585)	9.0 (63)
October	13.8 (669)	9.7 (77)
November	12.8 (618)	9.5 (70)
December	4.1 (199)	7.1 (60)
missing	0.1 (2)	0 (0)
Region		
South	38.0 (1,842)	-
Middle	25.8 (1,251)	-
North	26.2 (1,269)	-
Scotland	10.0 (485)	-
missing	0.02 (1)	-
Depressive symptoms, %(N)		
No	92.2 (4,471)	-
Yes	7.5 (361)	-
missing	0.3 (16)	-
Education, % (N)*		
	5.6 (272)	53.3 (394)
	12.3 (598)	19.5 (144)
	26.6 (1,289)	26.3 (194)
	16.9 (821)	
	26.6 (1,287)	
missing	12.0 (581)	1.0 (7)

*1958BC categories: none, <O-level, O-level, A-level or Higher;
PIVUS categories: Primary school <9 years, Secondary school 9-12 years or university level >12 years

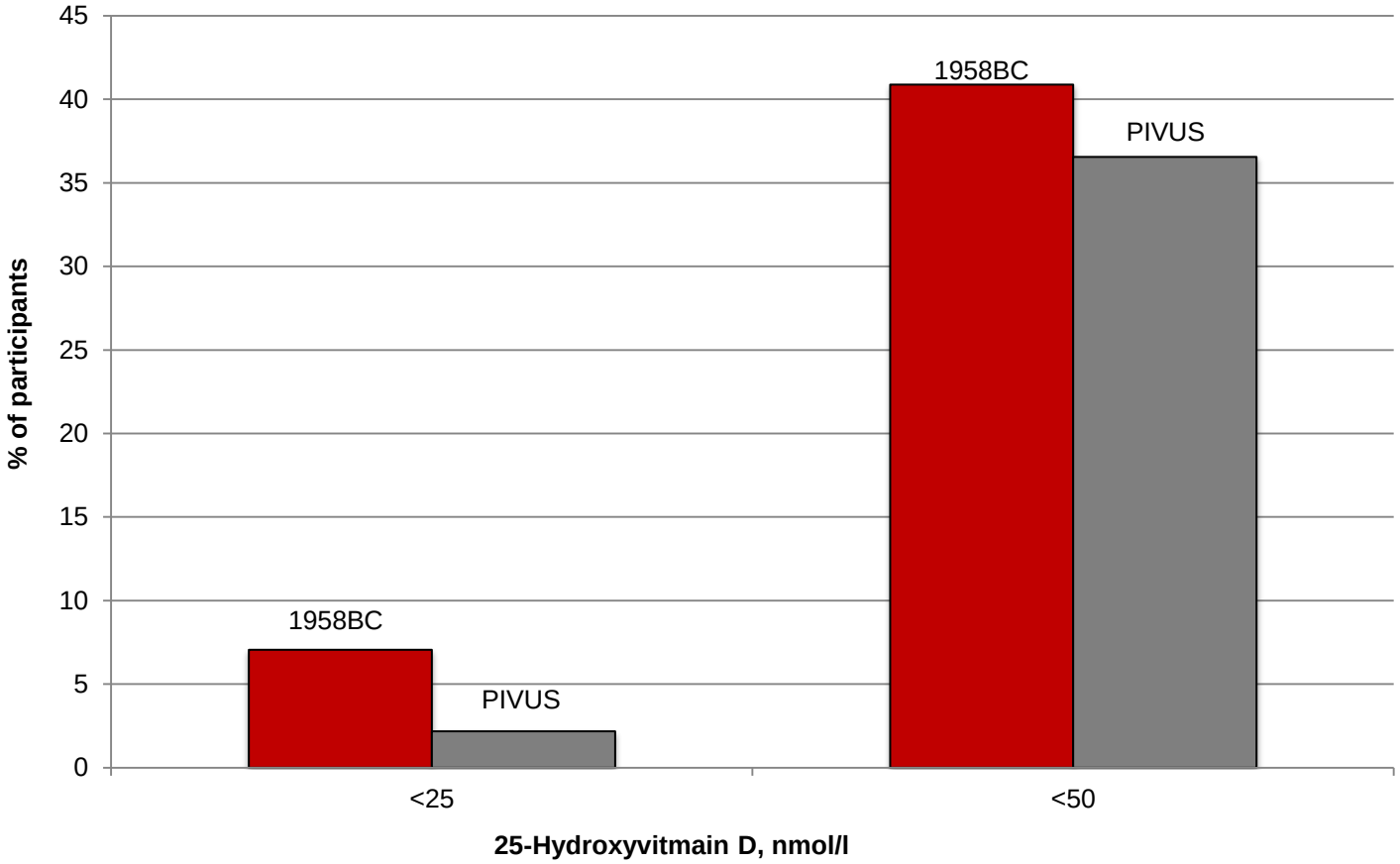


Figure 7.2: Percentage of participants with <25nmol/l and <50nmol/l 25(OH)D

1958BC: *n*=4,644; PIVUS: *n* =736

The distribution of *APOE* ε4 alleles in the two cohorts was similar, with two ε4 alleles being the least and two ε3 alleles the most prevalent (**Table 7.3**). The distribution of *APOE* ε4 alleles did not vary by gender in either cohort (p>0.67).

Table 7.3: Distribution of *APOE* ε4 alleles

	1958BC	**PIVUS**
APOE, % (N)		
zero ε4 alleles*	57.5 (2,787)	58.9 (435)
one ε4 alleles	23.9 (1,158)	24.1 (178)
two ε4 alleles	2.9 (139)	2.4 (18)
missing	15.8 (764)	14.6 (108)

*two ε3 alleles

Table 7.4 displays the mean and range of each of these individual tests. Women performed significantly better than men in all tests except the recall with reminder in PIVUS (p<0.001, for all tests in both cohorts).

Table 7.4: Mean performance on individual memory tests

	N	Mean (sd)	range
1958BC			
immediate word recall	4,848	6.6 (1.5)	0-10
delayed word recall	4,848	5.4 (1.8)	0-10
PIVUS			
recall, no reminder	739	9.6 (2.5)	0-16
recall, with reminder	739	6.0 (2.3)	0-14

7.3.2 Observational association between 25(OH)D and memory

There was a non-linear relationship between 25(OH)D and cognitive function in 1958BC, but this was not replicated in PIVUS (**Table 7.5**).

Table 7.5: Association between 25(OH)D and standardised memory function score

		Standardised memory function score			
		Model 1*		Model 2†	
25-hydroxyvitamin D, nmol/l	N(%)	Coefficient	(95% CI)	Coefficient	(95% CI)
1958BC					
<25	328 (7.1)	Reference	Reference	Reference	Reference
25-49	1,570 (33.8)	0.06	(-0.07 to 0.18)	0.04	(-0.10 to 0.17)
50-74	1,732 (37.3)	0.10	(-0.03 to 0.23)	0.10	(-0.04 to 0.24)
≥75	1,014 (21.8)	0.001	(-0.14 to 0.14)	0.01	(-0.14 to 0.17)
		P_{trend} = 0.71		P_{trend} = 0.83	
		$P_{curvature}$ = 0.01		$P_{curvature}$ = 0.02	
PIVUS					
<25	16 (2.2)	Reference	Reference	Reference	Reference
25-49	253 (34.4)	0.18	(-0.35 to 0.70)	0.22	(-0.32 to 0.75)
50-74	328 (44.6)	0.19	(-0.34 to 0.71)	0.22	(-0.30 to 0.75)
≥75	139 (18.9)	0.28	(-0.26 to 0.82)	0.32	(-0.23 to 0.86)
		P_{trend} = 0.31		P_{trend} = 0.29	
		$P_{curvature}$ = 0.55		$P_{curvature}$ =0.42	

Coefficient from linear regression model;

* adjusted for age (PIVUS) gender, month of blood collection, region (in 1958BC), education and depressive symptoms (in 1958BC): *n*=4,068 for 1958BC, *n*=729 for PIVUS.

† adjusted for age (PIVUS), gender, month, region (in 1958BC), education and depressive symptoms (in 1958BC) and number of *APOE* ε4 alleles: *n*=3,426 for 1958BC, *n*=623 for PIVUS

7.3.3 APOE ε4 associations

APOE ε4 alleles and 25(OH)D

In the 1958BC, average 25(OH)D concentrations were the highest for individuals with two ε4 alleles, however this association was not statistically significant following adjustment for gender, month of blood collection, and region (p_{trend}=0.56). Conversely, in PIVUS, average 25(OH)D concentrations were slightly higher for individuals with two ε3 alleles, however, this was not statistically significant (p_{trend}=0.27 after adjusting for gender and month of blood collection) (**Figure 7.3** and **Appendix 5.4**). Further adjustment for memory did not affect the coefficients in the model (**Appendix 5.4**).

APOE ε4 alleles and memory function

In the 1958BC, individuals with one or two ε4 alleles had lower memory scores compared with those with two ε3 alleles, however this association was not statistically significant following adjustment for gender, region, education and depressive symptoms (p_{trend}=0.17). In PIVUS, memory function was found to be significantly lower in the presence of ε4 alleles (p_{trend}=0.001). The coefficients in these models did not change following adjustment for 25(OH)D concentrations, indicating that the association between *APOE* ε4 alleles and memory is independent of 25(OH)D concentrations (**Table 7.6**).

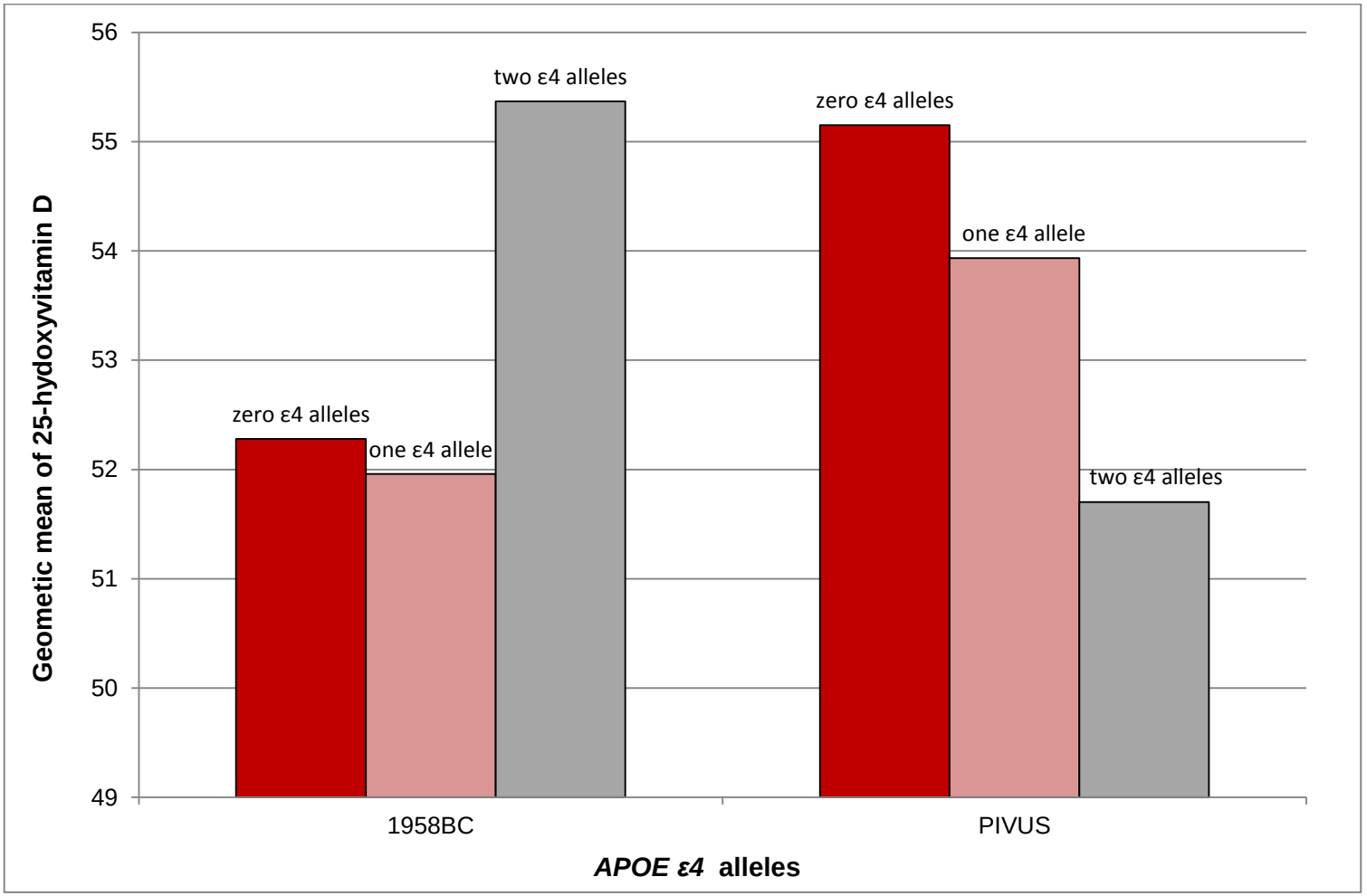


Figure 7.3: Geometric mean of 25(OH)D according to number of *APOE* ε4 alleles

Table 7.6: Association between *APOE* ε4 and memory score

		Standardised memory scores			
		Model 1*		Model 2†	
	N(%)	Coefficient	(95% CI)	Coefficient	(95% CI)
1958BC					
zero *APOE* ε4 alleles‡	2,787 (68.2)	Reference	Reference	Reference	Reference
one *APOE* ε4 alleles	1,158 (28.4)	-0.05	(-0.12, 0.02)	-0.05	(-0.12, 0.02)
two *APOE* ε4 alleles	139 (3.4)	-0.04	(-0.21, 0.13)	-0.04	(-0.21, 0.13)
		p_{trend} = 0.17		p_{trend} = 0.18	
PIVUS					
zero *APOE* ε4 alleles‡	549 (68.5)	Reference	Reference	Reference	Reference
one *APOE* ε4 alleles	229 (28.6)	-0.25	(-0.42, -0.08)	-0.25	(-0.42, -0.07)
two *APOE* ε4 alleles	24 (3.0)	-0.44	(-0.92, 0.03)	-0.45	(-0.92, 0.03)
		p_{trend} = 0.001		p_{trend} = 0.001	

*adjusted for age (in PIVUS), gender, region (for 1958BC), education and depressive symptoms (in 1958BC):*n*=3,578 for 1958BC, *n*=625 for PIVUS;

†adjusted for age (in PIVUS), gender, region (for 1958BC), education, depressive symptoms (in 1958BC) and 25(OH)D concentrations: *n*=3,426 for 1958BC, *n*=623 for PIVUS

‡ refers to two ε3 alleles

7.3.4 GxE interaction

1958BC

Three-way interaction between 25(OH)D, 25(OH)D^2 and number of *APOE* ε4 alleles was not significant (overall $p_{interaction}$=0.75, **Appendix 5.5**). However the curvature term (25(OH)D^2) remained significant in this GxE model (*p*=0.02). Therefore, a two-way GxE model was examined, adjusting for the curvature term.

The two-way GxE, adjusting for the curvature term was significant (overall $p_{interaction}$=0.02, **Figure 7.4**).

PIVUS

The three-way interaction was not significant (overall $p_{interaction}$=0.87, **Appendix 5.5**). Although the curvature term was not significant in this model (*p*=0.31), a two-way GxE model, adjusting for the curvature term was applied to be comparable with GxE models of the 1958BC. A GxE interaction was found using the adjusted two-way interaction model (overall $p_{interaction}$=0.02, **Figure 7.5**).

The association between 25(OH)D and memory function, stratified by number of *APOE* ε4 alleles in both cohorts is displayed in **Appendix 5.6**.

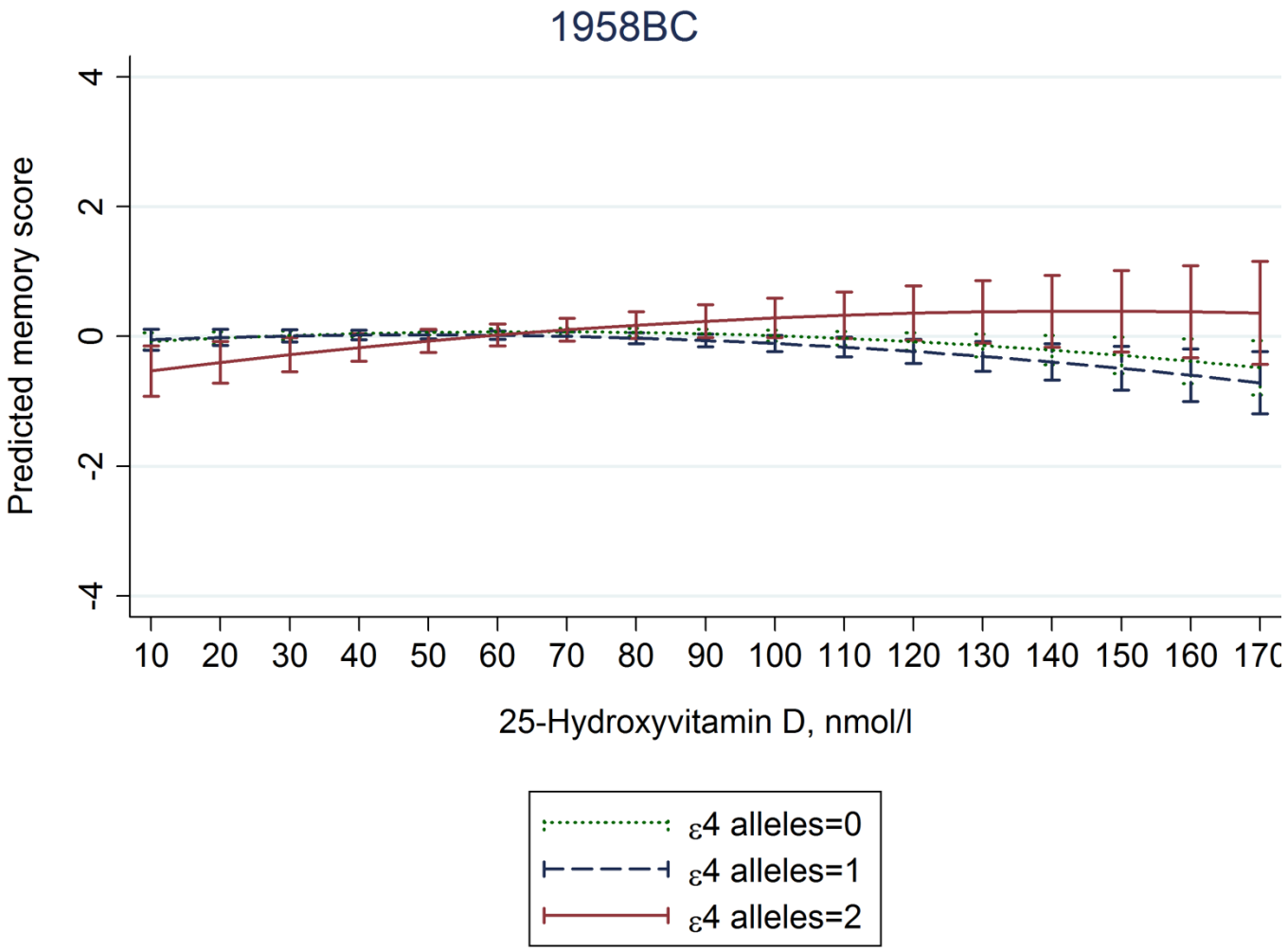


Figure 7.4. Gene-environment interaction in 1958BC

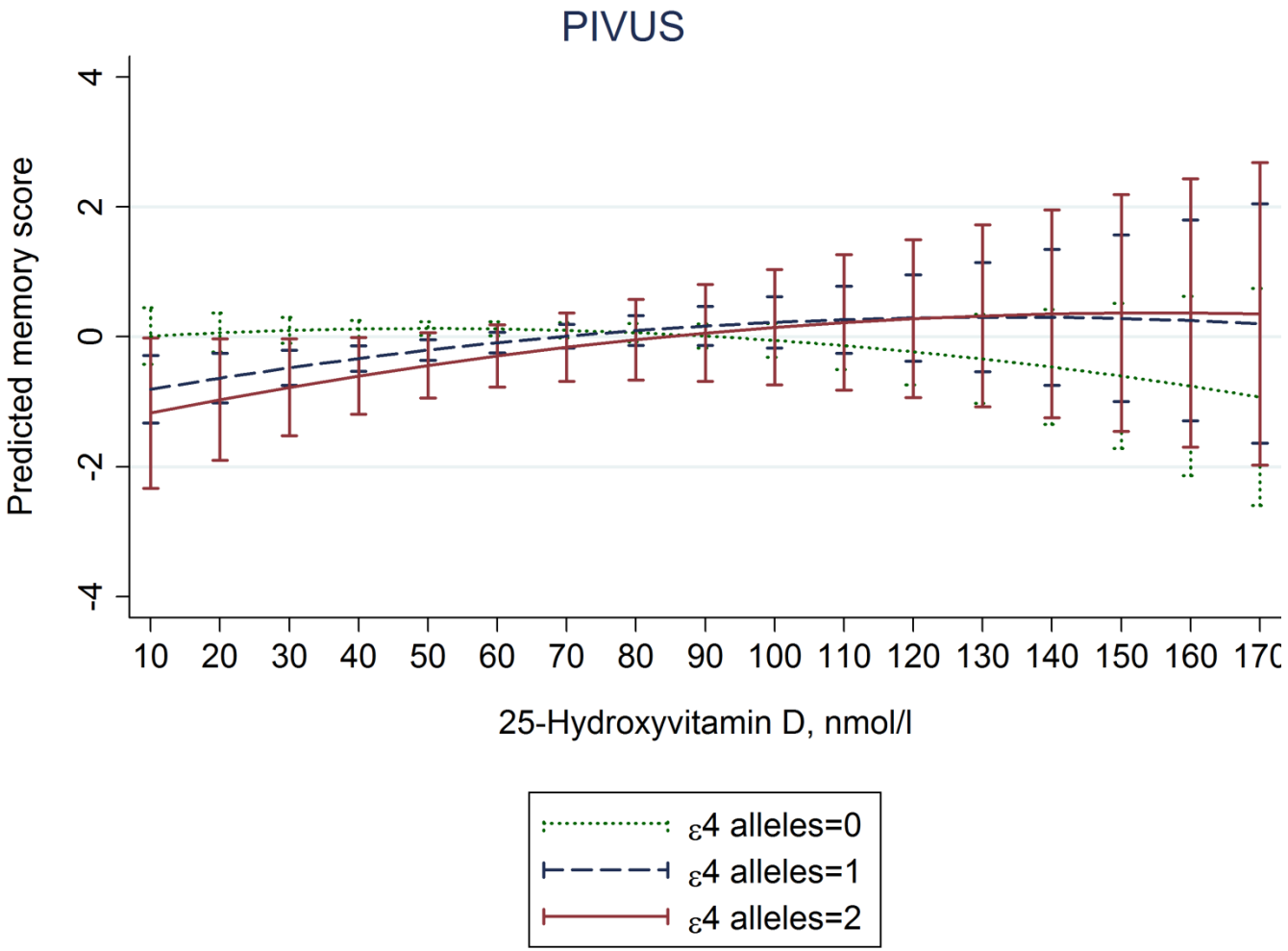


Figure 7.5: Gene-environment interaction in PIVUS

7.3.5 Additional analyses

Recessive models

In the 1958BC GxE model (**Figure 7.4**), the greatest difference was between those with two *APOE* ε4 alleles compared with others, suggesting a recessive genetic model may be appropriate. Using a recessive model, at lower 25(OH)D concentrations, those with two *APOE* ε4 alleles perform poorly on memory tests, however at higher 25(OH)D concentrations they score higher on memory tests ($p_{interaction}$=0.01 in the recessive model, **Figure 7.6**).

In PIVUS, the greatest difference seemed to be between those with one or two *APOE* ε4 alleles compared with individuals with two *ε*3 alleles. The recessive GxE model was not significant in PIVUS ($p_{interaction}$=0.24 in the recessive model, **Figure 7.7**).

Stratified analyses using these recessive models in both cohorts are given in **Appendix 5.7.**

Sensitivity analyses

There was no difference in results when restricting analyses to those with information on 25(OH)D and memory function with no *APOE* ε2 alleles (**Appendices 5.8** to **5.11**).

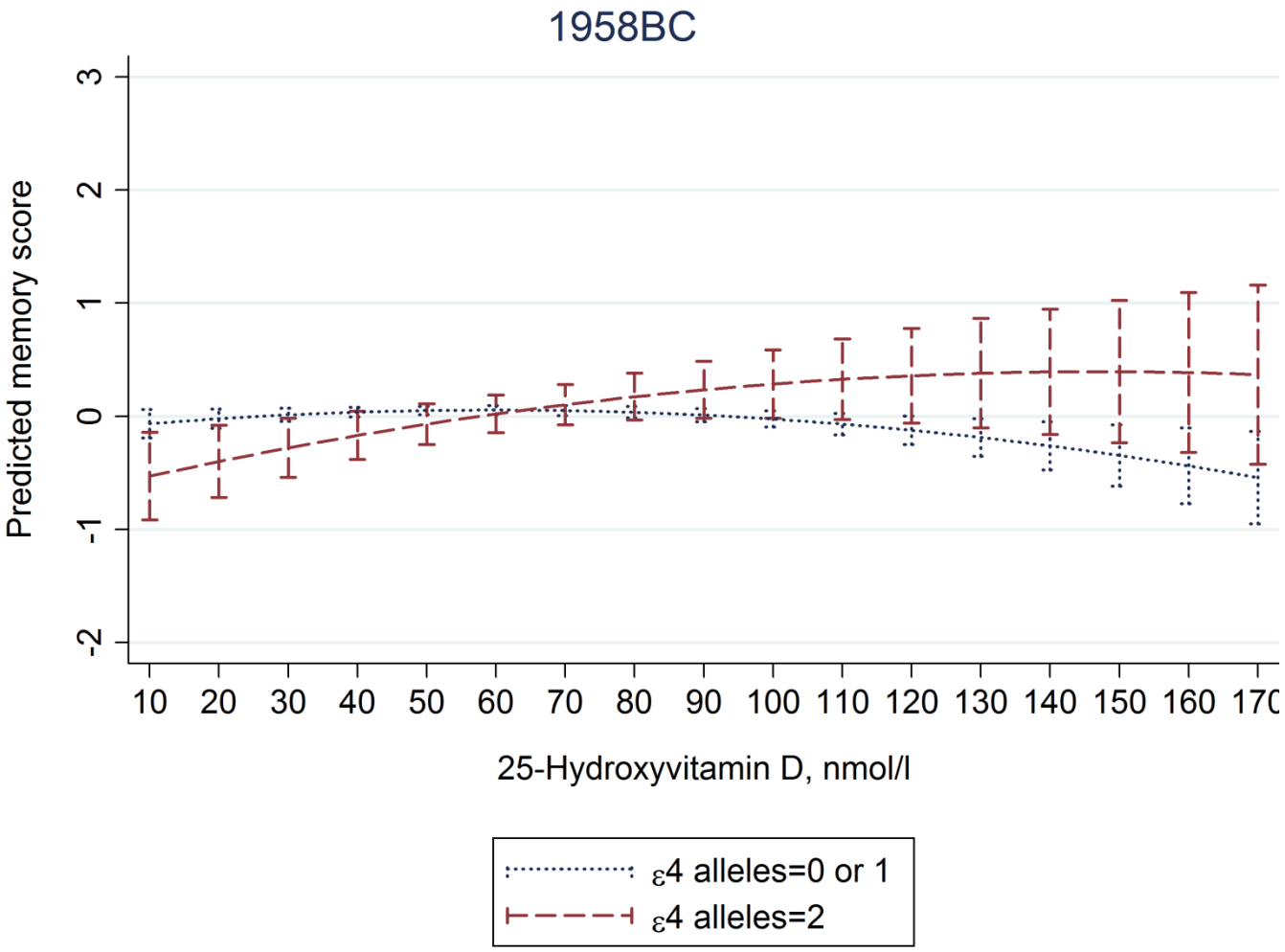


Figure 7.6: Gene-environment interaction in 1958BC, using recessive model

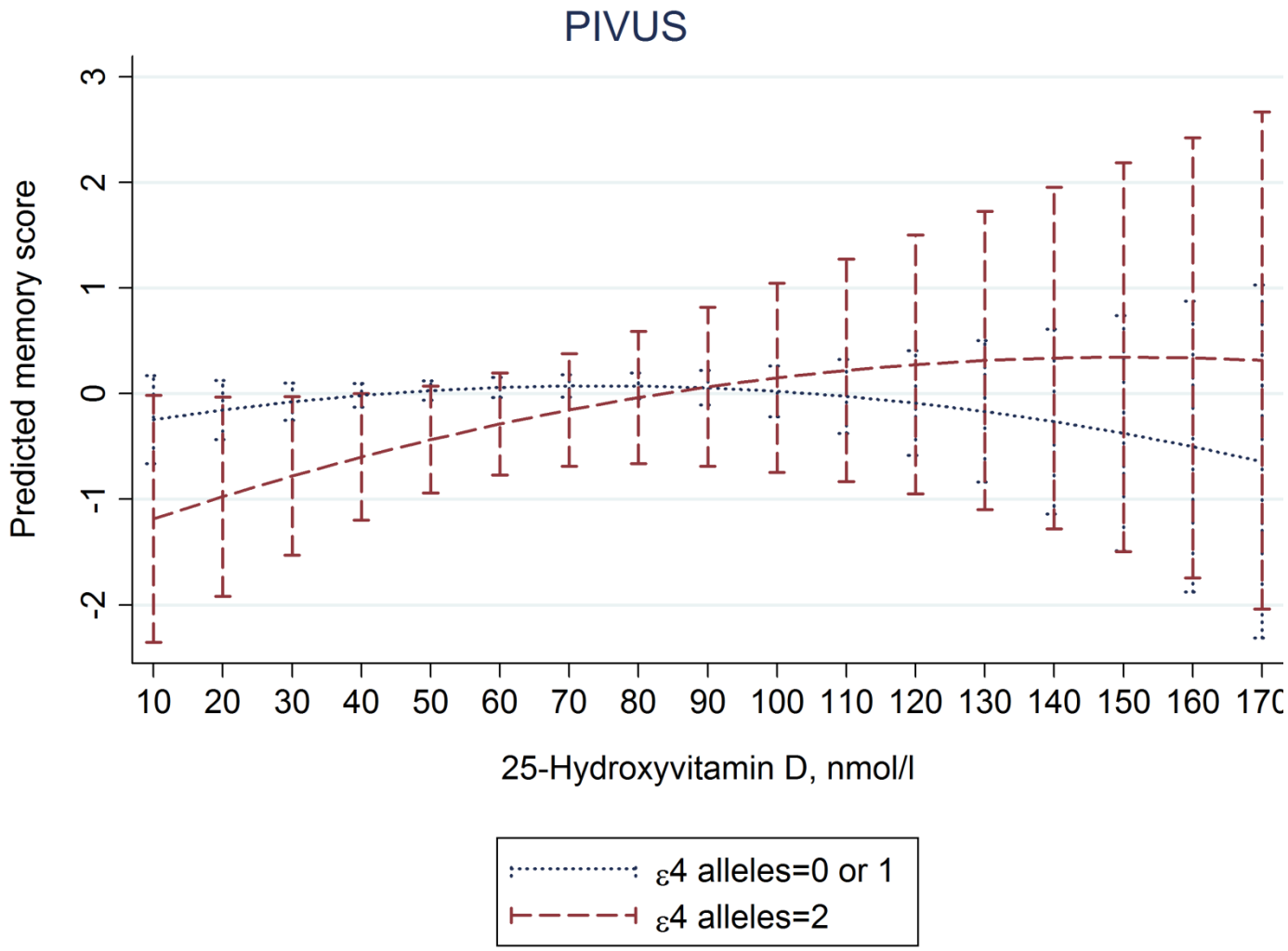


Figure 7.7: Gene-environment interaction in PIVUS, using recessive model

7.4 Discussion

The purpose of this study was to examine if the non-linear relationship between 25(OH)D and memory function (**Chapter 6**), and in particular the suggested poor memory function in individuals with the highest concentrations, is affected by *APOE* genotype. A paper related to findings in this study can be found in **Appendix 1.3**.

The non-linear association between 25(OH)D and memory in 1958BC (here and in **Chapter 6**) did not replicate in PIVUS. Inconsistent findings between 1958BC and PIVUS could be due to differences in the distribution of 25(OH)D concentrations between the cohorts. Mean 25(OH)D concentrations in PIVUS were higher compared with 1958BC and there were fewer individuals at the extremes of the distribution (i.e. 2.4% had <25nmol/l while 3.1% had ≥100nmol/l), possibly reducing the ability to detect associations between 25(OH)D and memory amongst participants with high and low 25(OH)D concentrations.

Associations between *APOE* ε4 alleles and 25(OH)D were not significant in either 1958BC or PIVUS and they were in opposing directions. While the direction of the relationship between *APOE* ε4 alleles and higher 25(OH)D concentrations in 1958BC is consistent with previous studies, the association between *APOE* ε4 and lower 25(OH)D in PIVUS is not (40). The opposing trends between *APOE* ε4 alleles and 25(OH)D in the two cohorts may be affected by cohort differences and/or methodological issues. For example, participants of 1958BC are aged 50 years while individuals from PIVUS are aged 70 years, which could indicate that the relationship between *APOE* ε4 alleles and 25(OH)D may be age-dependent. Furthermore, despite the similar distribution of *APOE* ε4 alleles across the two cohorts, there is a limited number of participants with two *APOE* ε4 alleles in PIVUS (*n*=24) which may have resulted in a reduction in power.

The association between *APOE* ε4 alleles and reduced memory function was weaker and not significant in 1958BC compared with PIVUS. This observation suggests that the association between *APOE* ε4 alleles and reduced memory

function may be stronger amongst older people. Findings were consistent with a previous study indicating that the *APOE* ε4 allele is not associated with reduced intelligence in females aged 19-21 years (424). Similarly, results were supportive of studies showing no significant association between the *APOE* ε4 allele and lower performance on cognitive tests amongst middle aged adults (425, 426). However, in contrast to our findings, another study found that the *APOE* ε4 allele is associated with lower measures of learning and memory tasks amongst middle-aged adults (24-60 years) (427). One potential difficulty in assessing memory function is the inability to ascertain if the effects of *APOE* ε4 genotype on memory function in mid-adulthood are independent of any pre-clinical signs of dementia.

Evidence for the discrete association of *APOE* ε4 alleles with 25(OH)D and memory in 1958BC and PIVUS remains uncertain. However, the presence of a GxE interaction may mask direct effects and could notably alter the observed associations (194). Findings from GxE analyses suggest that increasing 25(OH)D concentrations would be particularly beneficial for homozygous *APOE* ε4 carriers in 1958BC, while both heterozygous and homozygous *APOE* ε4 carriers in PIVUS may benefit from increasing 25(OH)D concentrations. The presence of an age-dependent interaction could explain the slightly different interaction results between the two cohorts. For example, in mid-adulthood, the discerning effect of *APOE* ε4 alleles on the relationship between 25(OH)D and memory may be particularly noticeable amongst those with two alleles of *APOE* ε4. The influence of *APOE* ε4 on the relationship between 25(OH)D and memory may increase with age causing the presence of any *APOE* ε4 to affect the association.

There are relatively few robust and replicable examples of GxE interactions in the literature and even fewer examining *APOE* and 25(OH)D. To the best of my knowledge, this study displays novel findings of effect modification by *APOE* ε4 alleles on the association between 25(OH)D and memory. In line with this study, there is some evidence of effect modification by *APOE* genotype on the association of certain lifestyle and health factors with cognitive function. Kang et al. examined 70-80 year old participants of the Nurse's Health Study and found the risk of cognitive decline was particularly high amongst *APOE* ε4

carriers who have untreated hypertension (198). However, Brown et al found no evidence of interaction between *APOE* ε4 and homocysteine, folate, B6 or B12 in predicting cognitive function amongst older adults (70-79 years) (428). Kang et al took a similar approach to coding *APOE* ε4 as the presented study i.e. excluding ε2 carriers from analyses. However, Brown et al coded *APOE* ε4 as either carriers or non-carriers with no reference to treatment of ε2 carriers. Since there is some suggestion that those with an *APOE* ε2 allele may perform better on memory tests (429, 430), the exclusion of ε2 carriers may be beneficial in examining the independent actions of ε4.

Interestingly, a study using participants who were in a similar age group to the 1958BC (<64 years), found no association between *APOE* ε4 and any measured cognitive domain nor did it display any interaction effects with certain risk factors (i.e. head trauma and alcohol abuse) (426). However, in a 4-year follow-up to this study, *APOE* ε4 was associated with lower cognitive function, as measured by the Mini-Mental State Examination, and there was evidence for interaction effects with low education and previous head trauma (431). This suggests that the modifying effect of *APOE* genotype may become more pronounced when participants are older.

Interpretation of the results may be limited as there were differences in how the memory tests in 1958BC and PIVUS were measured and mean concentrations on 25(OH)D were higher and deficiency and insufficiency prevalence was lower in in PIVUS compared with 1958BC. Additionally, there are relatively small numbers of participants with two *APOE* ε4 which may have reduced the ability to detect differences in this group. Precision could have been affected by missing information on 25(OH)D concentrations, although it is unlikely that missing data introduced bias since those with missing 25(OH)D measurements were similar to the remaining sample. Furthermore, there was no difference in results when restricting analyses to participants with complete data on 25(OH)D. Causality cannot be determined as we cannot rule out the possibility of unmeasured confounding despite adjustment for gender, month of blood collection, region of residence, education and depressive symptoms.

7.4.1 Conclusion

Results from this chapter suggest that, for memory, increasing 25(OH)D concentrations would be particularly beneficial for those with *APOE* ε4 alleles. The possibility to improve cognitive outcomes in genetically susceptible individuals who carry two *APOE* e4 alleles is intriguing; however, these novel findings clearly require independent replication to consolidate the evidence before randomised controlled trials to examine these effects are warranted.

7.5 Summary

- The aim of this chapter was examine if *APOE ε4* genotype contributes to the association between 25(OH)D and memory function
- There was evidence of interaction between *APOE* ε4 and 25(OH)D in 1958BC and PIVUS suggesting the non-linear relationship between 25(OH)D and memory function is most pronounced in those with *APOE* ε4 alleles compared with two ε3 alleles
- This GxE interaction study suggests that the relationship between 25(OH)D and memory function may be modified by the presence of *APOE* ε4 alleles. However, this interaction may be age dependent and requires replication in larger, independent studies across many age groups

Chapter 8 Genetic study: Mendelian randomisation to assess vitamin D and cognitive function

8.1 Introduction

Findings from observational study of the 1958 British birth cohort (1958BC) suggest there is a non-linear association between 25(OH)D and memory function (**Chapter 6**) which could be subject to effect modification by *APOE* ε4 alleles (**Chapter 7**).

Well-conducted observational studies may allude to an association between an exposure and outcome, however their inherent limitations can obscure the nature of the relationship between 25(OH)D and cognitive function through unmeasured confounding and the possibility of reverse causality (**Chapter 3**).

Mendelian Randomisation (MR) is an approach that uses a genetic variant (associated with the exposure of interest) to estimate the causal relationship between an exposure and outcome (**Figures 3.4** and **3.5**). As described in **Chapter 3**, this method can help to overcome some limitations of observational studies as it relies on the random assignment of genetic variants from parents to offspring to reduce the possibility of confounding (432). Furthermore, since the genetic variant is established at birth, the possibility of reverse causality is minimised (432).

The causal relationship between 25(OH)D and cognitive function will be evaluated using MR analyses, whereby genetic variants (in this case, single nucleotide polymorphisms, SNPs) are used as a proxy for lifelong 25(OH)D status (**Chapter 2**).

8.2 Data and methods

8.2.1 Participants

MR studies generally require large sample sizes to capture the small variation in the exposure that can be attributed to the genetic variant (**Chapters 3** and **4**). If the sample size is too small, a real effect may not be detected. To increase the sample size, data were obtained from nine European cohorts (**Chapter 4**):

1) 1958 British birth cohort (1958BC)
2) Austria Stroke Prevention Survey (ASPS)
3) English Longitudinal Study of Ageing (ELSA)
4) Epidemiologische Studie zu Chancen der Verhütung, Früherkennung und optimierten Therapie chronischer Erkrankungen in der älteren Bevölkerung (ESTHER)
5) Helsinki Birth Cohort Study (HBCS)
6) The Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS)
7) The Tromsø Study (Tromsø)
8) Uppsala Longitudinal Study of Adult Men (ULSAM)
9) Whitehall II (WHII)

Data was restricted to participants with information on at least one genetic variant, at least one cognitive outcome and who had data relating to 25(OH)D concentrations (**Chapter 4** for further details). Three cohorts (ASPS, ELSA and WHII) that did not have information on 25(OH)D concentrations were restricted to participants with data on genetic variants and cognitive outcomes. In order to minimise the possibility of population stratification (see **Chapter 3** for definition), participants from non-European ancestry were excluded. Further exclusions for HBCS included those with a history of stroke (n=107) and those with a discrepancy between reported gender and gender determined by their genotype (n=8).

Table 8.1 outlines the sample size, number of participants with available data on the genetic variants, 25(OH)D, cognitive outcomes and mean age at the time of cognitive testing.

Table 8.1: Eligible participants for MR study

	Participants	***Year of cognitive test***	***Mean age (SD) at cognitive test***	***DHCR7***	***CYP2R1***	**25-hydroxyvitamin D**	**Memory**	**Global cognition**
1958BC	6,346	2008	50 (0)	6,212	5,752	5,890	6,260	6,151
ASPS	826	1994-2003	65.6 (8.0)	826	826	0	780	740
ELSA	5,532	2002	66.1 (9.7)	5,476	5,483	0	5,477	5,361
ESTHER	9,749	2005-2008	74.0 (2.8)	8,312	8,296	9,502	1,694	1,694
HBCS	1,811	2001-2003	68.1 (2.9)	1,629	1,630	1,035	1,017	1,017
PIVUS	1,010	2006-2011	70.2 (0.2)	986	901	999	794	768
Tromsø	12,336	2001	64.5 (9.9)	11,806	9,404	7,161	4,898	4,605
ULSAM	1,194	1991-1995	71.0 (0.6)	1,123	1,124	1,194	0	858
WHII	5,150	2002-2004	60.9 (6.0)	4,510	4,501	0	5,073	5,043
Total	43,954			40,743	37,694	25,781	25,997	26,281

8.2.2 Variables

Genetic variants

The genetic variants used in this chapter are detailed in **Chapter 4**. Briefly, *DHCR7* and *CYP2R1* were found to be effective instruments for MR studies on vitamin D, particularly when they are combined into a synthesis score. The synthesis score has been found to explain more variation in 25(OH)D concentrations and was more robust to the MR assumptions compared with individual genetic variants (207).

A description of the genotyping techniques used in each cohort to obtain these genetic variants is described in **Chapter 4**. Quality checks of each genetic variant were conducted following data exclusions. The call rate, minor allele frequency and Hardy Weinberg equilibrium for the genetic variants in each study are reported in **Appendix 6.1**. The minor allele frequencies were compared with HapMap data on populations with a European ancestry[7] and were found to be approximately similar.

For analysis purposes, both *DHCR7* and *CYP2R1* genotypes were coded as 0-2 depending on the presence of alleles associated with increasing 25(OH)D concentrations. In this case, the homozygous genotypes with two vitamin D-increasing alleles were coded as 2. The synthesis score was created using *DHCR7* and *CYP2R1* genotypes by summing them on the basis of their alleles (207). The few participants with 0 and 1 alleles associated with increasing 25(OH)D concentrations (ranging from 9.6% in 1958BC to 17.4% in Tromsø) were grouped together.

Exposure: 25-hydroxyvitamin D

Information on 25(OH)D was available in six cohorts (**Table 8.1**). Details of how 25(OH)D concentrations were measured in each cohort can be found in **Chapter 4**. In Tromsø, 25(OH)D concentrations had been shown to be overestimated in smokers, therefore models with 25(OH)D analysed in Tromsø were controlled for smoking status (433). In ESTHER, 25(OH)D concentrations

[7] http://hapmap.ncbi.nlm.nih.gov/cgi-perl/gbrowse/hapmap27_B36/#search

of 780 women with 0 to 29.5nmol/l were recorded as 29.54nmol/l due to the lower detection limit of the Diasorin assay employed to measure the serum sample in 2001. The assay used for men did not have this detection limit. Additionally, in ESTHER, those with >250nmol/l (*n*=6) were excluded from analyses. No 25(OH)D measurements were available for participants of ASPS, ELSA or WHII.

Distributions of 25(OH)D in each cohort can be seen in **Appendix 6.2**. Since 25(OH)D was found to be skewed, natural log (ln) transformation was applied to approximate a normal distribution. In order to examine the analyses stratified by 25(OH)D concentrations, gender and study specific 25(OH)D tertiles were created, where each tertile contains a third of the cohort's eligible participants (**Table 8.2**).

Outcomes: Global and memory cognitive function

Cognitive tests in each cohort were segregated into a standardised memory or global cognitive domain (**Chapter 4**). **Table 8.3** illustrates how cognitive tests were grouped and **Appendix 6.3** presents the distribution of tests in each cohort.

Covariates

MR analyses are based on the assumption that potential confounders are distributed equally between individuals with and without the effect allele, similar to the randomisation process in a randomised controlled trial (**Chapter 3**). Therefore, the number of covariates included are limited to those that have been shown to be strongly associated with cognitive function and/or 25(OH)D concentrations. The main covariates include; gender, age (in years) (434), month of blood collection for 25(OH)D, education (434-436) and depressive symptoms (437-439). A description of how education and depressive symptoms were measured in each cohort is given in **Table 4.7**.

Table 8.2: Gender and study specific 25(OH)D tertiles in each cohort

	Gender-specific 25(OH)D tertile 1, nmol/l					**Gender-specific 25(OH)D tertile 2, nmol/l**					**Gender-specific 25(OH)D tertile 2, nmol/l**				
Study	N	mean	SD	min	max	N	mean	SD	min	max	N	mean	SD	min	max
1958BC	1,881	31.8	8.1	9.5	44.8	1,996	55.3	6.5	43.4	66.5	2,013	86.5	17.3	66.5	187.3
ESTHER	3,117	31.0	5.1	7.0	41.7	3,178	46.5	7.1	35.9	63.6	3,207	74.8	21.9	49.3	225.6
HBCS	340	42.7	7.1	19.0	53.0	345	60.1	4.7	51.0	69.0	350	83.3	18.6	67.0	292.0
PIVUS	332	37.4	7.3	10.9	49.7	326	55.9	5.2	45.0	66.0	341	79.1	14.4	61.0	143.0
Tromsø	2,374	38.5	7.8	10.1	50.0	2,390	56.9	4.7	48.2	65.2	2,397	81.1	14.7	65.3	192.2
ULSAM	398	48.6	9.4	5.0	59.9	398	68.1	4.8	60.0	76.4	398	89.4	12.4	76.4	153.3

Table 8.3: Categorisation of cognitive tests in each cohort

Cohort	Memory Domain	Global Cognition
1958BC	Immediate word recall Delayed word recall	Immediate word recall Delayed word recall Verbal fluency Speed of processing
ASPS	Word association Digit association Story recall Trail recall Design recall Wisconsin card sorting test Alters-Konzentrations-Test TMT-B Digit-span backward Digit-span forward Complex reaction time task	Word association Digit association Story recall Trail recall Design recall
ELSA	Immediate word recall Delayed word recall	Immediate word recall Delayed word recall Verbal fluency Speed of processing Orientation in time
ESTHER	Verbal short-term memory Working memory Verbal long-term memory	Verbal short-term memory Working memory Verbal long-term memory Verbal fluency Inductive reasoning
HBCS	Immediate verbal recall Delayed verbal recall Verbal learning	Immediate verbal recall Delayed verbal recall Verbal learning Verbal fluency
PIVUS	7MS: word recall with reminder 7MS: word recall without reminder	7MS: word recall with reminder 7MS: word recall without reminder 7MS: clock drawing 7MS: verbal fluency 7MS: orientation in time TMT-A TMT-B MMSE
Tromsø	12-word memory test	12-word memory test Finger tapping test Digit symbol coding test
ULSAM	NA	MMSE TMT-A TMT-B
WHII	Memory test score	Memory test score Alice-Heim 4 Mill Hill Score Verbal fluency (phonemic) Verbal fluency (semantic)

Global cognition: Derived variable to capture the full range of cognitive measures captured in the cohorts. MMSE: Mini-mental state examination; 7MS: 7-minute screen test; TMT: Trail making test

In 1958BC, MR models using 25(OH)D were additionally controlled for region of residence at 46 years (categorised as Southern England and Channel Islands (South), Middle England and Wales (Middle), Northern England and Isle of Man (North) or Scotland). Models involving 25(OH)D analysed in Tromsø were also adjusted for smoking status (categorised as do not smoke or smoke daily).

Additional covariates from the 1958BC were used to investigate the suitability of the chosen genetic variants as instruments for MR analyses. These covariates included; region of residence (south or north), socioeconomic position (SEP) (I/II/IIINM or IIIM/IV/V), educational attainment (none/some/O-level/A-level or degree), depressive symptoms (yes/no), BMI (<30kg/m^2 or ≥30kg/m^2), alcohol consumption (non-/light/moderate drinker or heavy/very heavy drinker), smoking (yes or no), time spent outside (<3 hours/day or ≥3hours/day), time spent watching TV/using a PC (<3 hours/day or ≥3 hours/day), use of suncover (rarely or most of the time), vitamin D supplement (<one a day or ≥one a day) and oily fish consumption (<weekly or ≥weekly). A description of these variables can be found in **Chapter 4**.

8.2.3 Statistical methods

Chapter 3 provides detail on the MR approach and analyses. This section outlines additional information regarding the statistical methods used.

Analyses of observational data

Linear regression models were used to examine the association between 25(OH)D and the two measures of cognitive function in 1958BC, ESTHER, HBCS, PIVUS, Tromsø and one measure of cognitive function in ULSAM. In these analyses, naturally log-transformed 25(OH)D was included in the model as a continuous exposure and global and memory cognitive scores were the standardised outcomes. Estimates from these models were divided by 100 to reflect change in cognitive function for a 1% increase in 25(OH)D concentrations (346). These models were adjusted for age, gender, month of 25(OH)D blood collection, educational attainment and depressive symptoms. Analyses in 1958BC and Tromsø additionally controlled for region of residence and smoking status respectively.

Since findings from **Chapter 6** implied there was a non-linear association between 25(OH)D and cognitive function, presence of a significant curvature was investigated (**Chapter 3**). Effect modification was examined from the most complex models, which tests for interaction between gender or age, 25(OH)D and the curvature term of 25(OH)D (**Chapter 3**). Effect modification by age (grouped as <65 year and ≥65 years) was assessed in individual cohorts where possible. Observational analyses were stratified based on findings from the interaction analyses.

All analyses were conducted in individual cohorts with available data and combined into a meta-analysis. As specified in **Chapter 3**, random effects meta-analysis were run if the Q statistic indicated significant heterogeneity at the alpha level of 0.05. Further sensitivity analysis was conducted to account for potential heterogeneity not captured by the alpha level. There was no difference between fixed and random effects meta-analysis in any of the models examined.

MR instrument validation

A number of steps were taken to ensure the chosen genetic variants are suitable instruments for MR analyses.

a) The genetic variant's association with naturally log-transformed 25(OH)D was assessed in terms of both average increase in vitamin D-increasing allele and the influence of each additional allele compared with zero vitamin D-increasing alleles. The strength of the relationship between genetic variants with 25(OH)D was examined by calculating the F-statistic (**Chapter 3**). This investigation used coefficients obtained from meta-analyses which were weighted by study sample size.
b) The assumption of an independent association between the genetic variants and 25(OH)D was examined using data from the 1958BC (**Chapter 3** for more details). Covariates included; region of residence, SEP in adulthood, educational attainment, depressive symptoms, BMI, alcohol consumption, smoking status, time spent outside during the last month, time spent using PC or TV, use of suncover, use of vitamin D

supplements and consumption of oily fish. The association between genetic variants and 25(OH)D were adjusted for these covariates to examine independency.

c) The direct association between the genetic variants and covariates, adjusted for 25(OH)D were also examined. Bonferroni correction was applied to account for multiple testing (440). As there were 12 confounders, the p-value applied was <0.004 (i.e. 0.05/12).

d) Interaction between the potential confounders and genetic variants with naturally log-transformed 25(OH)D were investigated. Bonferroni correction was applied to account for multiple testing (i.e. 0.05/12).

e) To assess if the relationship between the genetic variants and 25(OH)D is non-linear, squared terms for *DHCR7* and *CYP2R1* on naturally log-transformed 25(OH)D were assessed in 1958BC.

MR analyses

The main steps for MR analyses are outlined in detail in **Chapter 3**. Firstly, the association of the genetic variants with naturally log-transformed 25(OH)D was assessed using linear regression models adjusted for age, gender, month of 25(OH)D assessment and additional adjustments were made for 1958BC (region of residence), Tromsø (smoking status) and HBCS (principal components to control for potential population stratification). The estimates from this analysis were multiplied by 100 to reflect percentage change in 25(OH)D per vitamin D-increasing allele (346). For consistency, the genetic variant association with 25(OH)D was stratified according to results from the observational interaction analyses.

Secondly, the association of genetic variants with cognitive function was assessed using linear regression models. ULSAM had information on global cognitive function only. These estimates reflect the change in cognitive function per vitamin D-increasing allele. These analyses were adjusted for age, gender, education and depressive symptoms in addition to principal components in HBCS. Analyses were stratified according to results from the observational interaction analyses.

As with observational analyses, MR analyses were conducted in individual cohorts with available data and combined into meta-analyses. There was no difference between fixed and random effects meta-analysis in the MR analyses.

Finally, the IV ratio was calculated using the meta-analysed coefficients examining 1) the association of genetic variants with 25(OH)D and 2) the association of genetic variants with cognitive function (**Chapter 3**).

Meta-regression

Meta-regression was used to examine heterogeneity amongst the cohorts using results from the meta-analysed observational analyses (441) (**Chapter 3**). Study characteristics that were hypothesised *a priori* to affect the association included gender, age group (<65 versus ≥65 years) and country region (categorised as UK (1958BC, ELSA and WHII), Europe (ASPS and ESTHER) and Nordic (HBCS, PIVUS, Tromsø and ULSAM).

8.3 Results

8.3.1 Descriptive results

The distribution of 25(OH)D and the main covariates can be seen in **Table 4.7.** Most cohorts have an equal distribution of men and women. However, ULSAM consists of an exclusively male population and WHII consists of mostly men (73.9%). All participants from the 1958BC are <65 years while participants from ESTHER, PIVUS and ULSAM are all ≥65 years. ASPS, ELSA, HBCS, Tromsø and WHII consist of participants in both age groups. The lowest mean 25(OH)D concentrations were found in ESTHER (46.8nmol/l) with the highest seen in ULSAM (65.8nmol/l).

The distribution of genetic variants in each cohort can be seen in **Appendix 6.4**. As expected, the percentage of participants was lowest for those with two copies of the 25(OH)D lowering allele in each cohort.

8.3.2 Observational association

The 1958BC, ESTHER, HBCS, PIVUS, Tromsø and ULSAM were eligible for inclusion in observational analyses as participants had data on both 25(OH)D and cognitive function.

Individual observational results between cohorts were heterogeneous (**Table 8.4**). In ESTHER, there was evidence for higher global and memory cognitive function per 10% increase in 25(OH)D concentrations (p<0.01 for both outcomes). In Tromsø, global cognitive function was found to be higher with increasing 25(OH)D concentrations (p=0.002), however the association with memory function was not significant (p=0.80). There was no linear association between 25(OH)D and either global or memory function in the remaining cohorts although the coefficients for HBCS and USLAM indicated lower cognitive function with higher 25(OH)D concentrations. The coefficients for global and memory cognitive function in 1958BC and PIVUS were in opposing directions.

There was a non-linear association between 25(OH)D and both global and memory cognitive function in 1958BC (p<0.04), however no significant curvature was found in the other cohorts (**Table 8.4**). There was no effect modification by gender or age-group (i.e. <65 versus ≥65 years) on the association between 25(OH)D and cognitive function in any cohort (**Table 8.4**).

Table 8.4: Association between and 25(OH)D and cognitive function

Cohort	Cognitive outcome	N	Coefficient, per 10% increase in 25(OH)D	(se)	p-value	$p_{curvature}$	$p_{gender\ interaction}$	$p_{age\ interaction}$ †
1958BC	Global cognition	5,028	0.002	(0.003)	0.57	0.04	0.41*	-
	Memory Cognition	5,125	-0.001	(0.003)	0.67	0.02	0.19*	-
ESTHER	Global cognition	1,553	0.02	(0.01)	0.00	0.89	0.35	-
	Memory Cognition	1,553	0.02	(0.01)	0.01	0.78	0.92	-
HBCS	Global cognition	725	-0.01	(0.01)	0.66	0.94	0.71	0.76
	Memory Cognition	725	-0.01	(0.01)	0.40	0.89	0.74	0.91
PIVUS	Global cognition	752	-0.004	(0.01)	0.73	0.26	0.30	-
	Memory Cognition	777	0.01	(0.01)	0.44	0.98	0.67	-
Tromsø	Global cognition	2,072	0.02	(0.01)	0.002	0.40	0.07	0.66
	Memory Cognition	2,193	0.002	(0.01)	0.80	0.13	0.42	0.27
ULSAM	Global cognition	858	-0.02	(0.01)	0.18	0.17	N/A	-

*p from 2-way interaction, adjusted for 25(OH)D[2]

†1958BC are all <65 years. ESTHER, PIVUS and ULSAM are all ≥65 years

The individual observational results examining the association of 25(OH)D with global and memory cognitive function were combined into meta-analyses in order to obtain pooled estimates. There was heterogeneity between the studies for global cognitive function (I^2=67.4%, $p_{heterogeneity}$=0.01), therefore a random-effects model was used. There was no significant heterogeneity between the studies for memory cognitive function (I^2= 46.2%, $p_{heterogeneity}$=0.12 for memory cognition) therefore, a fixed effects model was used in this case. There was no linear association between 25(OH)D and either global or memory cognitive function using these models (p>0.32 for both, **Figures 8.1 and 8.2**). However using a fixed effects meta-analyses (I^2=0.0%, $p_{heterogeneity}$≥0.48 for both outcomes), there was a non-linear association between 25(OH)D and both global and memory cognitive function ($p_{curvature}$<0.01 for both, **Figure 8.3**).

There was no effect modification by gender or age group on the association between 25(OH)D and either global or memory cognitive function (p≥0.49 for gender interaction and p≥0.29 for age-group interaction for both outcomes (**Appendices 6.5** and **6.6**).

Since there was a non-linear relationship between 25(OH)D and cognitive function, meta-analysed observational results were stratified by study-specific 25(OH)D tertiles (**Table 8.2**). When stratified by 25(OH)D tertiles, coefficients for global cognitive function per 10% increase in 25(OH)D were 0.01 (95% CI -0.001 to 0.03) in the lowest third (tertile 1 (T1)), 0.03 (95% CI 0.01 to 0.06) for T2 and -0.02 (95% CI -0.04, -0.004) for the highest third (T3). The coefficients for memory cognition per 10% increase in 25(OH)D were 0.003 (95% CI -0.01 to 0.02) for T1, 0.02 (95% CI -0.2 to 0.01) for T2 and -0.002 (95% CI -0.04 to -0.01) for T3 (**Figures 8.4** and **8.5**).

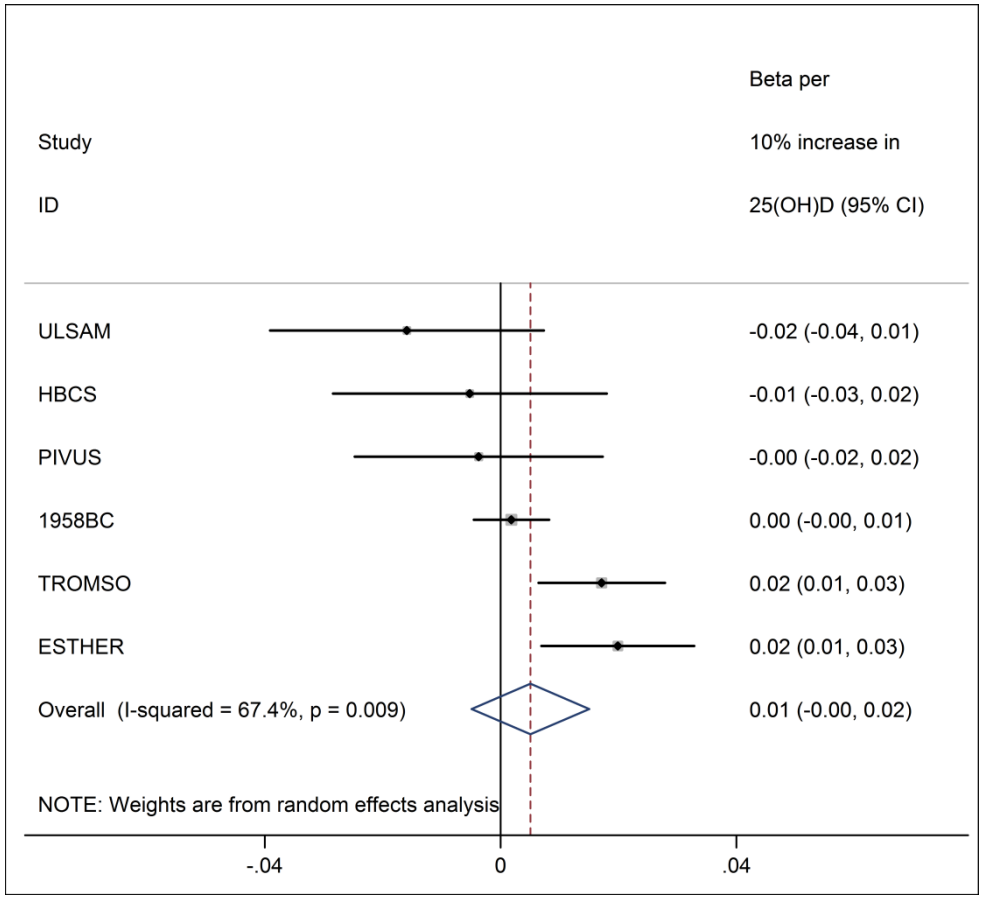


Figure 8.1: Association between 25(OH)D and global cognitive function

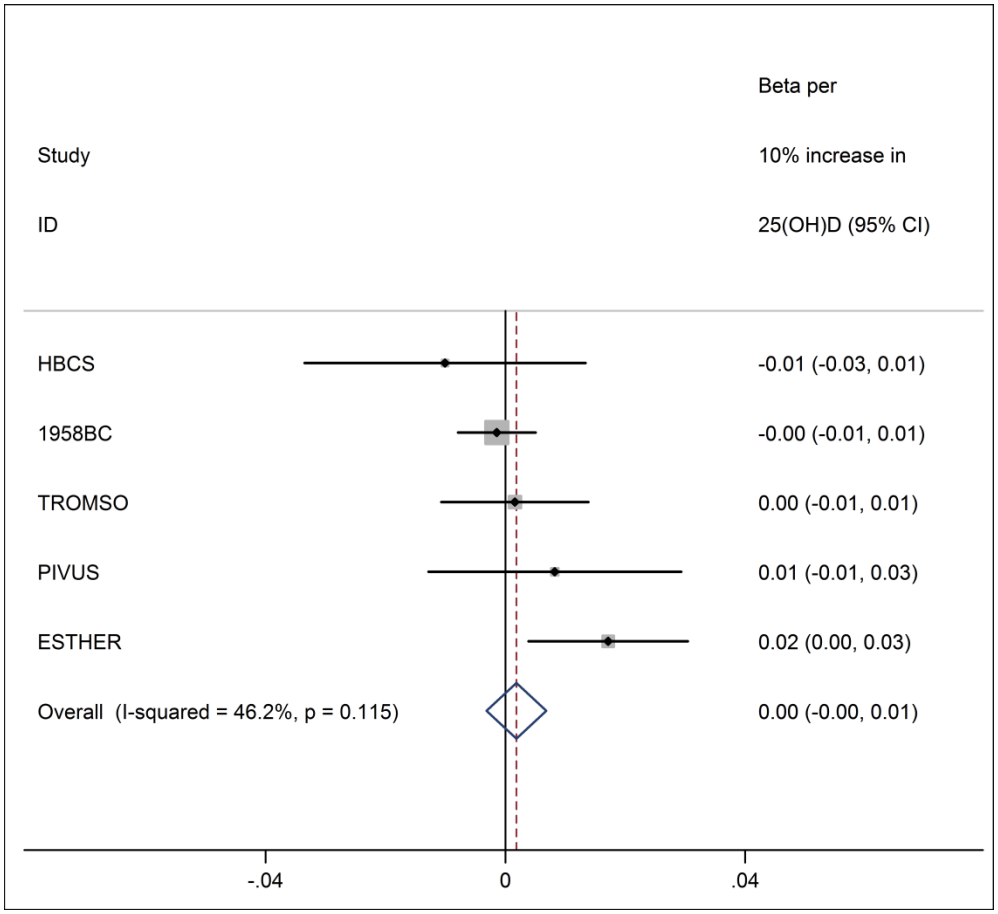


Figure 8.2: Association between 25(OH)D and memory function

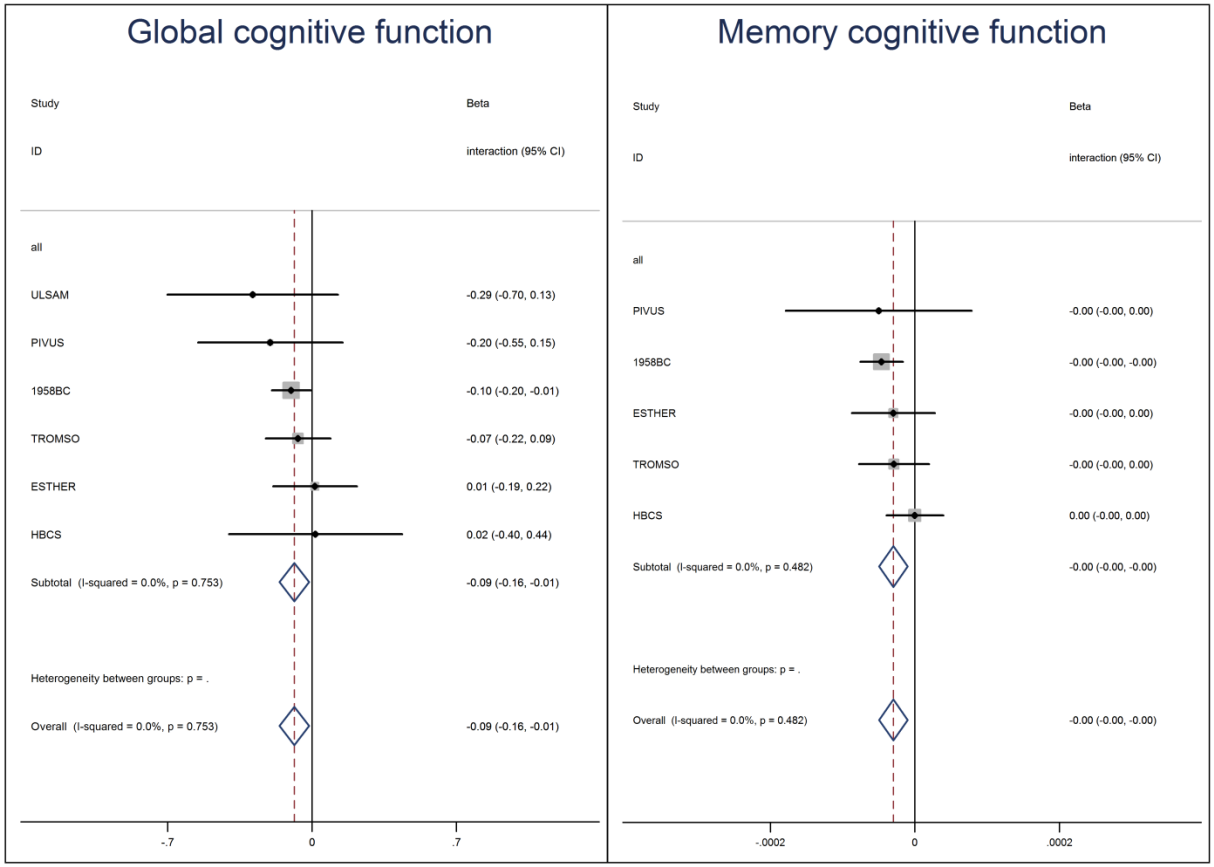


Figure 8.3: Non-linear association between 25(OH)D and cognitive function

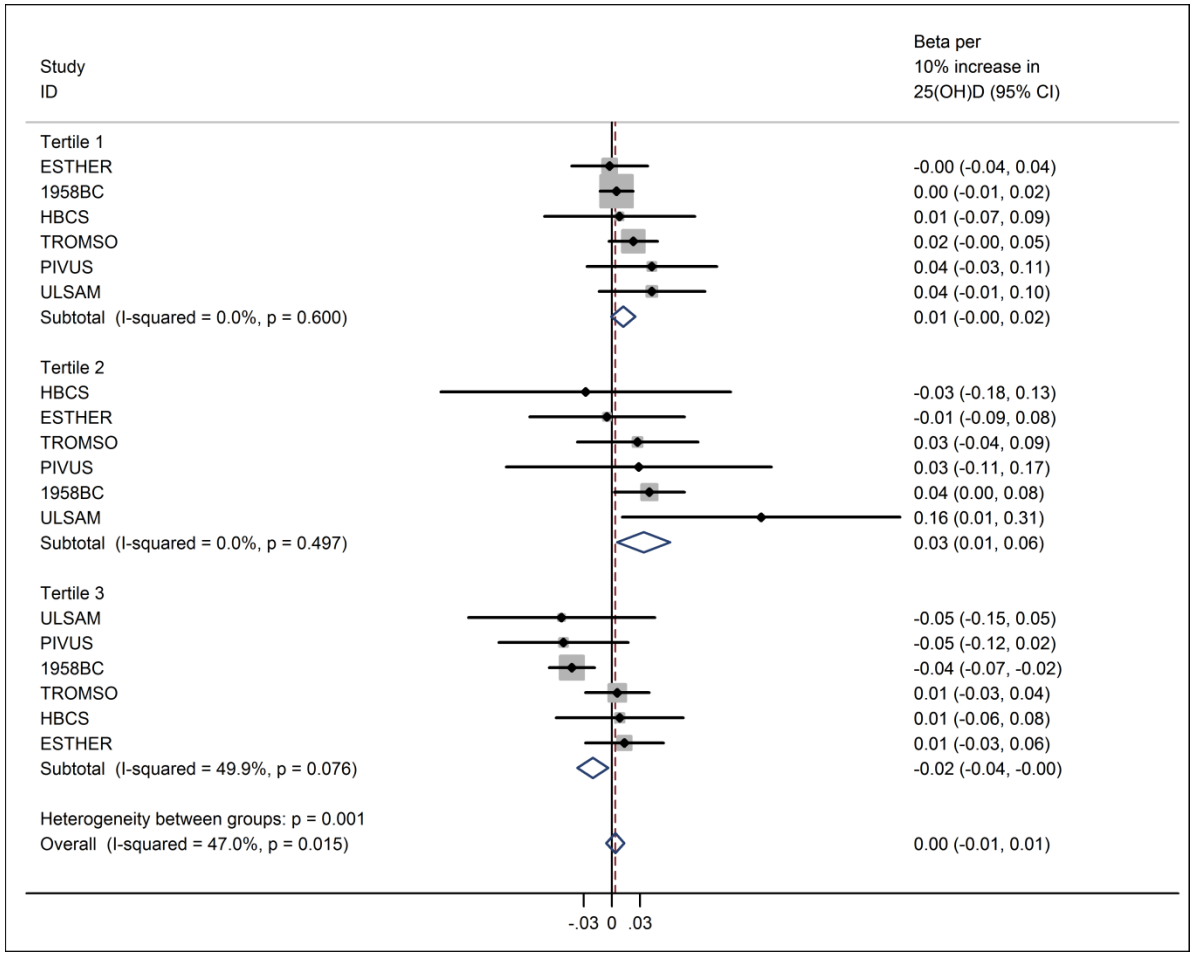


Figure 8.4: Association of 25(OH)D with global cognition: Stratified by 25(OH)D tertiles

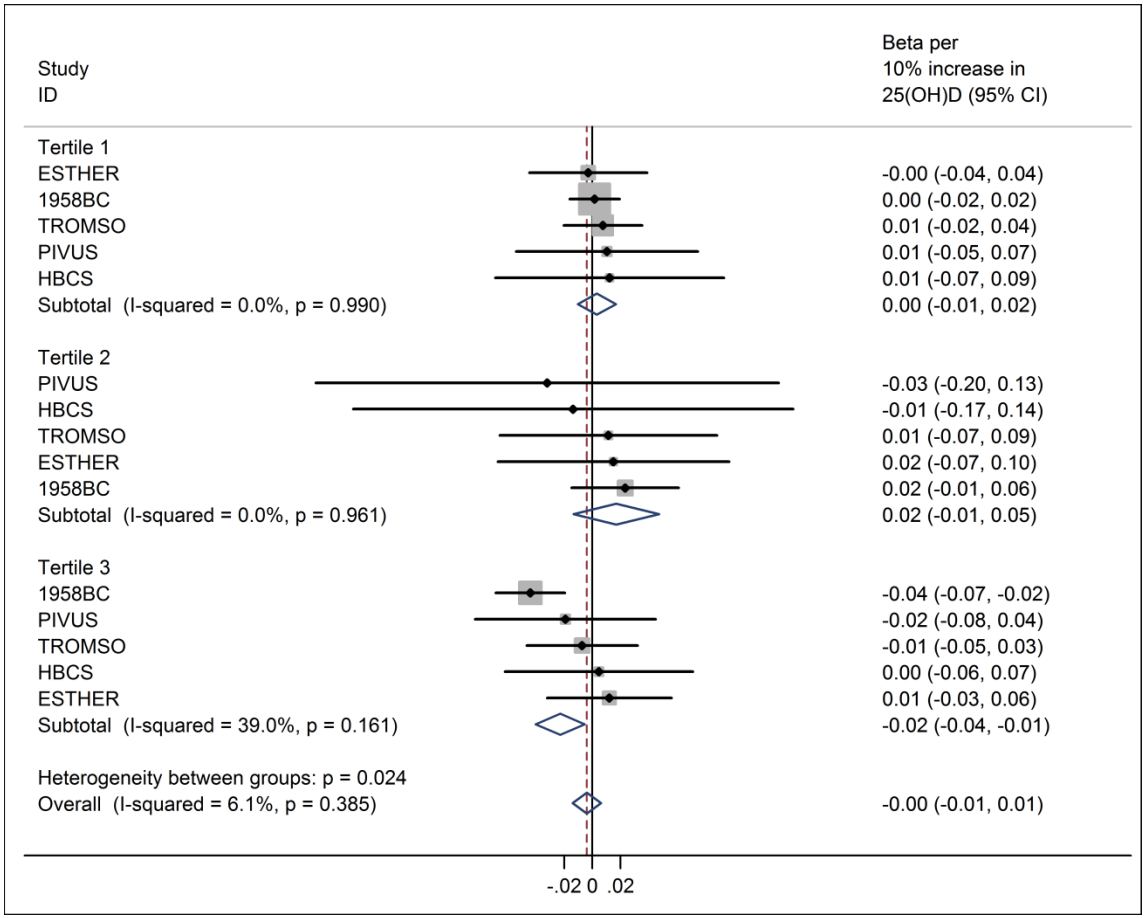


Figure 8.5: Association of 25(OH)D with memory function: Stratified by 25(OH)D tertiles

8.3.3 IV validation

To determine the suitability of the genetic variants for use in MR analysis, firstly, the strength of the genetic variant's association with 25(OH)D was assessed. **Figure 8.6** used fixed-effects meta-analyses to illustrate that the addition of each vitamin D-increasing allele is associated with greater increases in 25(OH)D concentrations. The weighted F-statistic was 31.42 for *DHCR7*, 49.19 for *CYP2R1* and 62.34 for the synthesis score. Since these F-statistics are >10, all genetic variants can be considered strong enough to use as a proxy for 25(OH)D in MR analyses (Staiger & Stock 1997).

The assumption of an independent association between genetic variants and 25(OH)D was examined using data from the 1958BC. The association between *CYP2R1* and synthesis score with 25(OH)D was not found to be sensitive to adjustment by lifestyle and dietary factors (**Figure 8.7**). However, the relationship between *DHCR7* and 25(OH)D was affected by dietary and lifestyle factors. This finding suggests that the association between *DHCR7* and 25(OH)D may not be independent of potential confounding factors.

Figure 8.8 shows the direct associations between genetic variants with these lifestyle and dietary factors. There was an association between *DHCR7* with region of residence and use of suncover following application of Bonferroni correction (correcting for 12 tests) and adjusting for 25(OH)D concentrations. *DHCR7* was associated with reduced odds of living in southerly regions and increased odds of using suncover most of the time.

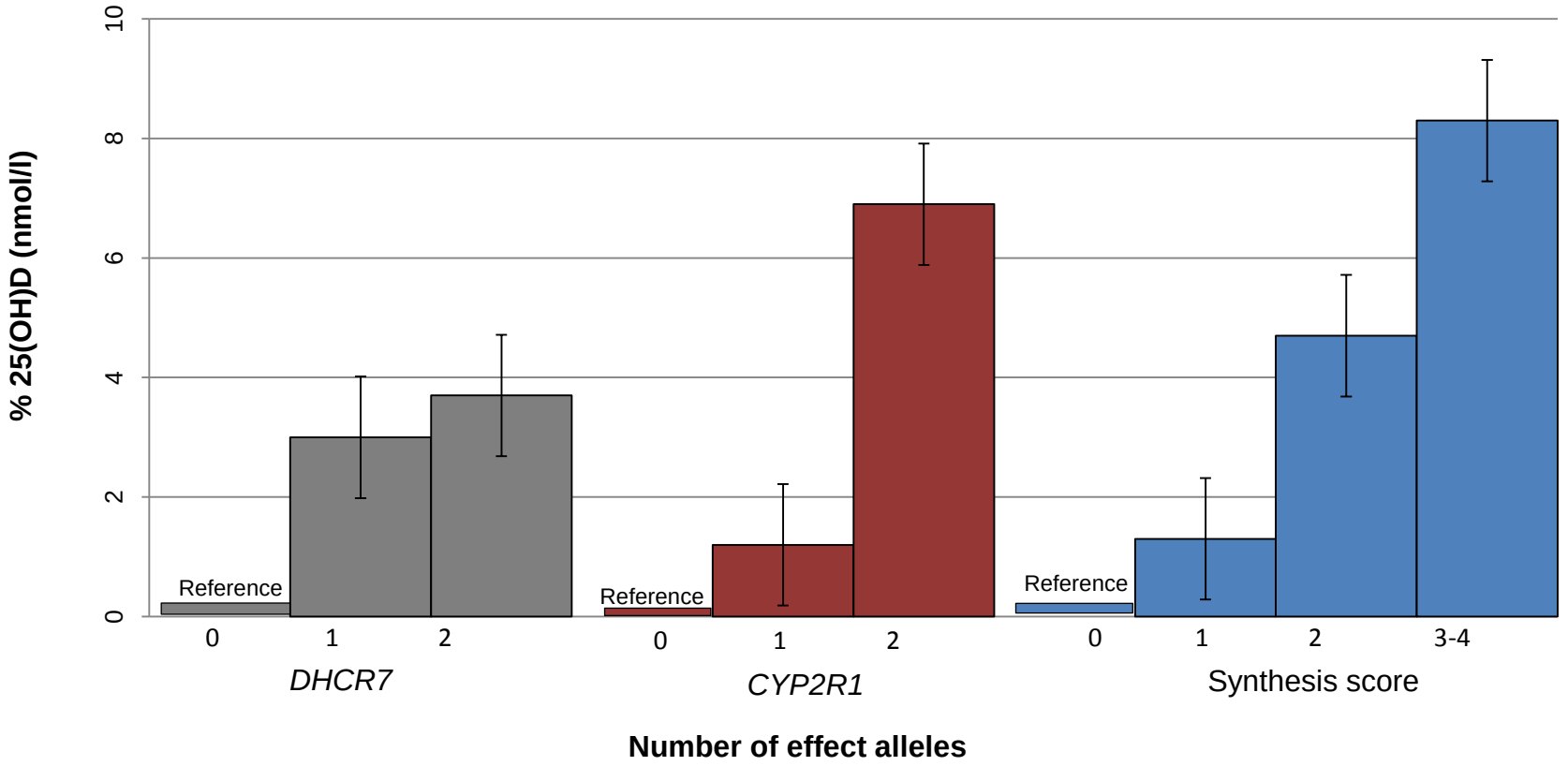


Figure 8.6: Association of effect alleles with 25(OH)D

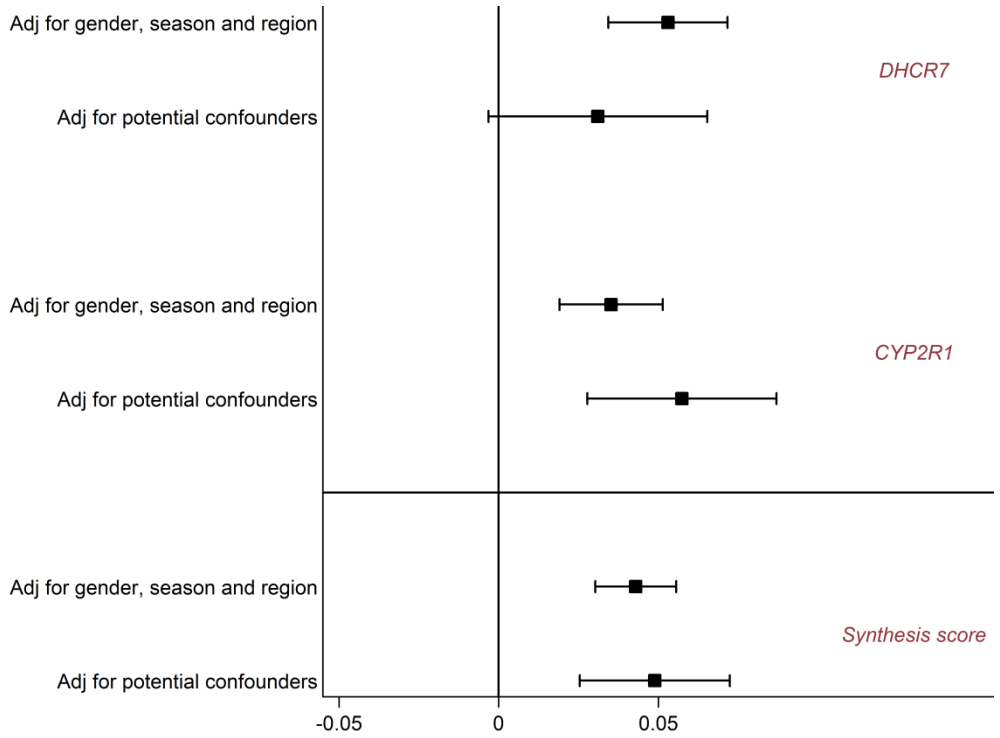


Figure 8.7: Association between genetic variants and 25(OH)D, adjusted for lifestyle factors

Cofounders include region of residence, socioeconomic position (SEP) in adulthood, educational attainment, depressive symptoms, BMI, alcohol consumption, smoking status, time spent outside during the last month, time spent using personal computer (PC) or television (TV), use of suncover, use of vitamin D supplements and consumption of oily fish

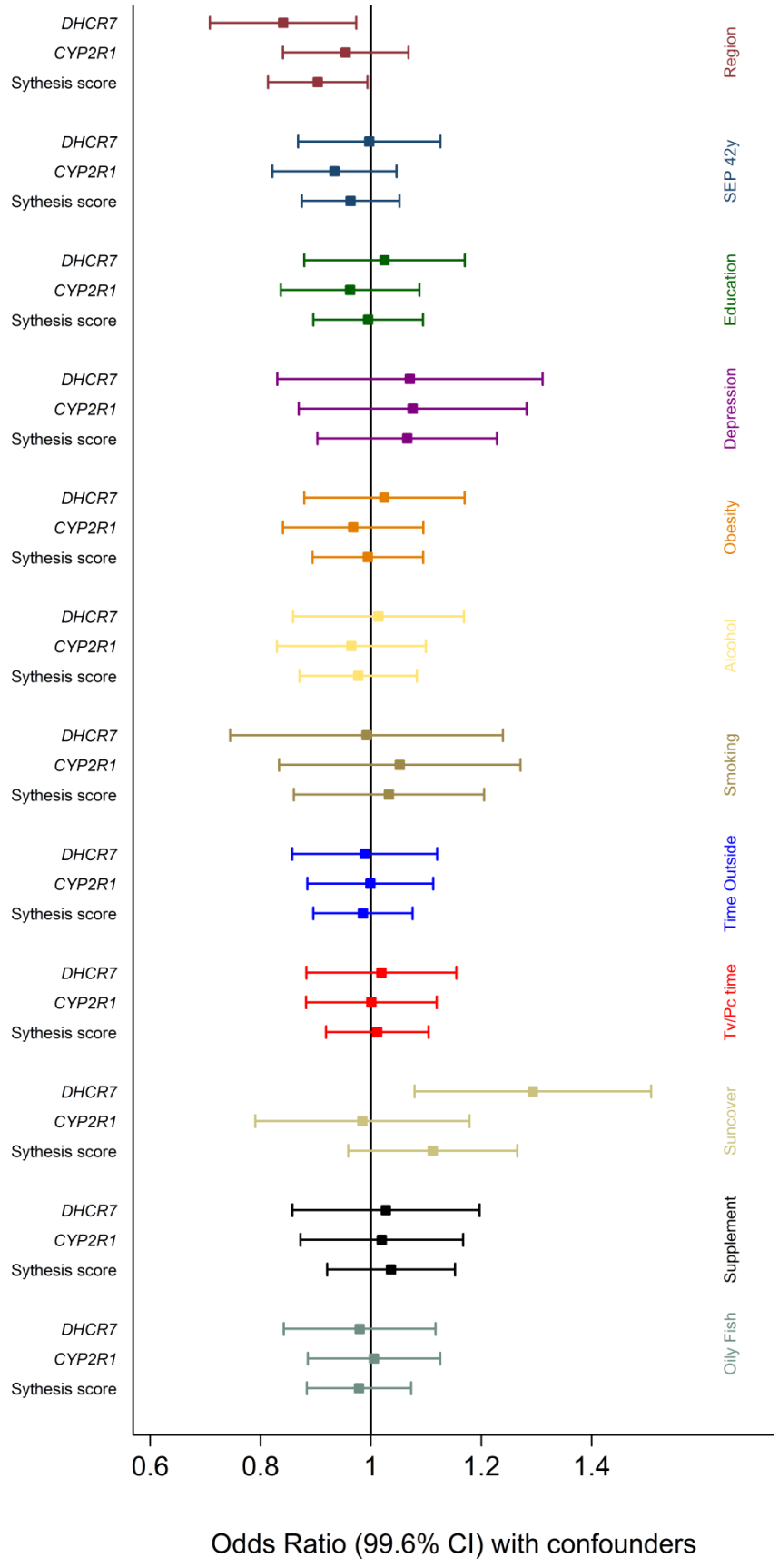


Figure 8.8: Association between genetic variants and potential confounders

There was no effect modification by these lifestyle and dietary factors on the association of genetic variants with 25(OH)D (Bonferroni corrected $p_{interaction} \geq 0.36$). There was no non-linear association between genetic variants with 25(OH)D (Bonferroni corrected $p_{curvature} \geq 0.60$).

8.3.4 Mendelian randomisation analyses

Genetic variant association with 25(OH)D

1958BC, ESTHER, HBCS, PIVUS, Tromsø and ULSAM were included when examining the association of genetic variants with 25(OH)D.

As expected, the 25(OH)D concentrations were higher per vitamin D-increasing allele across all cohorts except HBCS (**Table 8.5**)

These individual results were combined into meta-analyses, with a random effects model for *DHCR7* (I^2=73.7%, $p_{heterogeneity}$=0.002) and fixed effects models for *CYP2R1* and the synthesis score ($I^2 \leq 53.3\%$, $p_{heterogeneity} \geq 0.06$). 25(OH)D concentrations were higher per vitamin D-increasing allele of both genetic variants and the synthesis score (**Figures 8.9** to **8.11**). *DHCR7* showed 2.3% (95% CI 0.4% to 4.2%) higher 25(OH)D concentrations and *CYP2R1* showed 3.1% (95% CI 2.8% to 4.0%) higher 25(OH)D concentrations per vitamin D-increasing allele. The synthesis score indicated 2.9% (95% CI 2.2 to 3.5%) higher 25(OH)D concentrations per vitamin D-increasing allele.

Table 8.5: Association between genetic variants and 25-hydroxyvitamin D

Cohort	Genetic variant	N	Coefficient, % change in 25(OH)D per vitamin D-increasing allele	(se)	p-value
1958BC	*DHCR7*	5,773	5.34	(0.92)	<0.001
	CYP2R1	5,335	3.15	(0.79)	<0.001
	Synthesis score	5,238	4.08	(0.62)	<0.001
ESTHER	*DHCR7*	1,369	4.37	(1.55)	0.005
	CYP2R1	1,370	1.13	(1.38)	0.41
	Synthesis score	1,362	2.35	(1.04)	0.02
HBCS	*DHCR7*	707	-0.47	(1.70)	0.78
	CYP2R1	708	7.20	(1.61)	<0.001
	Synthesis score	707	3.70	(1.24)	0.003
PIVUS	*DHCR7*	981	1.03	(1.61)	0.52
	CYP2R1	896	4.93	(1.61)	0.002
	Synthesis score	887	3.30	(1.23)	0.012
Tromsø	*DHCR7*	3,336	1.13	(0.73)	0.12
	CYP2R1	3,351	2.42	(0.73)	0.001
	Synthesis score	3,320	2.01	(0.55)	<0.001
ULSAM	*DHCR7*	1,123	1.60	(1.32)	0.23
	CYP2R1	1,124	3.20	(1.30)	0.01
	Synthesis score	1,118	2.21	(0.97)	0.02

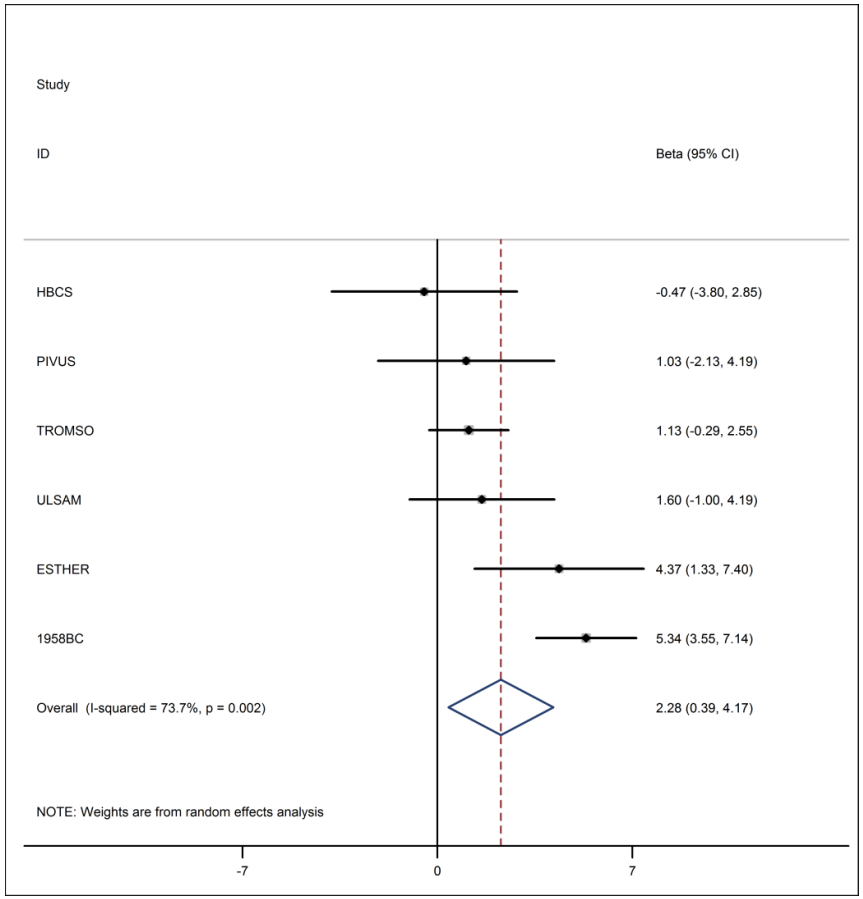


Figure 8.9: Association between *DHCR7* and 25(OH)D

Beta reflects % change in 25(OH)D per vitamin D-increasing allele

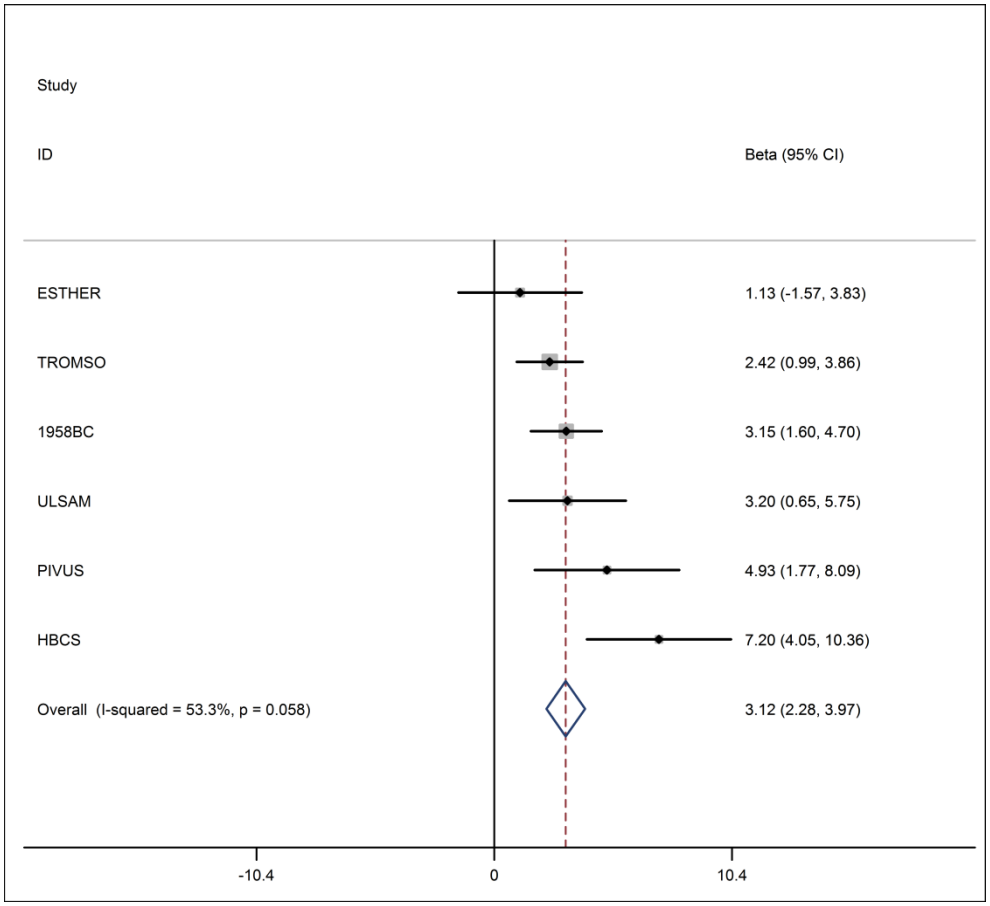


Figure 8.10: Association between *CYP2R1* and 25(OH)D

Beta reflects % change in 25(OH)D per vitamin D-increasing allele

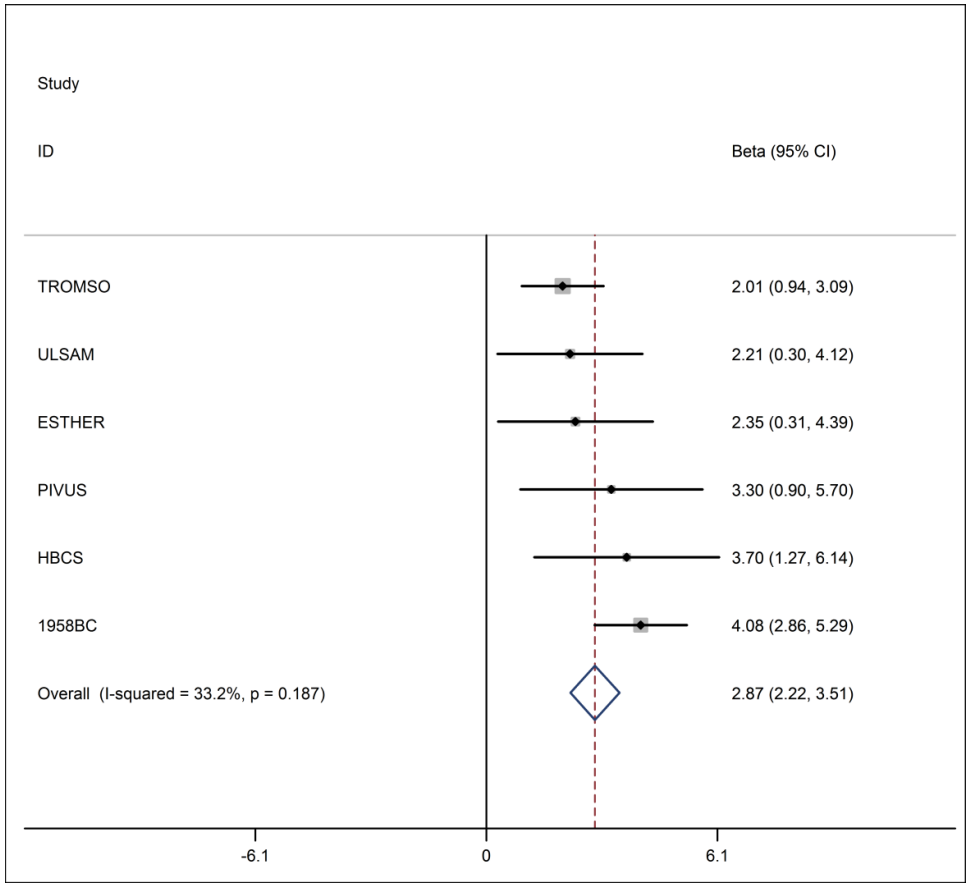


Figure 8.11: Association between synthesis score and 25(OH)D

Beta reflects % change in 25(OH)D per vitamin D-increasing allele

To be consistent with observational analyses, the association of the meta-analysed genetic variants with 25(OH)D were stratified by study-specific 25(OH)D tertiles (**Appendices 6.7** to **6.9**).

DHCR7 was associated with 1.5% higher (95% CI 0.4% to 2.5%) 25(OH)D concentrations per vitamin D-increasing allele in T1, 0.1% lower (95% CI 0.5% lower to 0.4% higher) 25(OH)D concentrations in T2 and 1.5% higher (95% CI 0.8% to 2.3%) concentrations in T3.

CYP2R1 showed 0.5% lower (95% CI 1.4% lower to 0.52% higher) 25(OH)D concentrations per vitamin D-increasing allele in T1, 0.1% lower (95% CI 0.3% lower to 0.5% higher) in T2 and 2.8% higher (95% CI 2.0% to 3.5%) in 25(OH)D concentrations in T3.

The synthesis score demonstrated 0.4% higher (95% CI 0.3% lower to 1.2% higher) 25(OH)D concentrations per vitamin D-increasing allele in T1, 0.01% higher (95% CI 0.3% lower to 0.3% higher) in T2 and 2.3% higher (95% CI 1.7% to 2.8%) in 25(OH)D concentrations per vitamin D-increasing allele in T3.

Genetic variant association with cognitive function

All cohorts were included when investigating the association of genetic variants with global and memory cognitive function. The results for each individual cohort can be found in **Appendix 6.10**.

There were no significant associations between the genetic variants or synthesis score with global or memory function in any of the cohorts. Furthermore, the direction of the relationship varied according to cohort. For example, the coefficient for 1958BC suggested higher global and memory cognitive function while WHII suggested lower cognitive function per vitamin D-increasing allele of the synthesis score.

These individual results were combined into fixed effects meta-analyses ($I^2 \leq 35.9\%$, $p_{heterogeneity} \geq 0.23$ for global cognitive function and $I^2 \leq 34.8\%$, $p_{heterogeneity} \geq 0.15$ for memory cognitive function). There was no association between either genetic variants or synthesis score with global cognitive function

(*p*≥0.08, **Figures 8.12** to **8.14**). *DHCR7* indicated a slightly higher memory cognitive function per vitamin D-increasing allele (*p*=0.04) however, there was no relationship between *CYP2R1* or the synthesis score with memory cognitive function (*p*≥0.29, **Figures 8.12** and **8.14**). Furthermore, the coefficients for both global and memory cognitive function were in the opposing direction for *DHCR7* and *CYP2R1*.

Table 8.6 displays results stratified by 25(OH)D tertiles. There was a trend for lower global cognitive function per vitamin D-increasing allele of *CYP2R1* in T2, however this was not significant in T1 or T3. The coefficients for *DHCR7* and synthesis score with global cognitive function across all 25(OH)D tertiles were in opposing directions and were not significant.

There was no association between either genetic variant or synthesis score with memory cognitive function across any of the 25(OH)D tertiles.

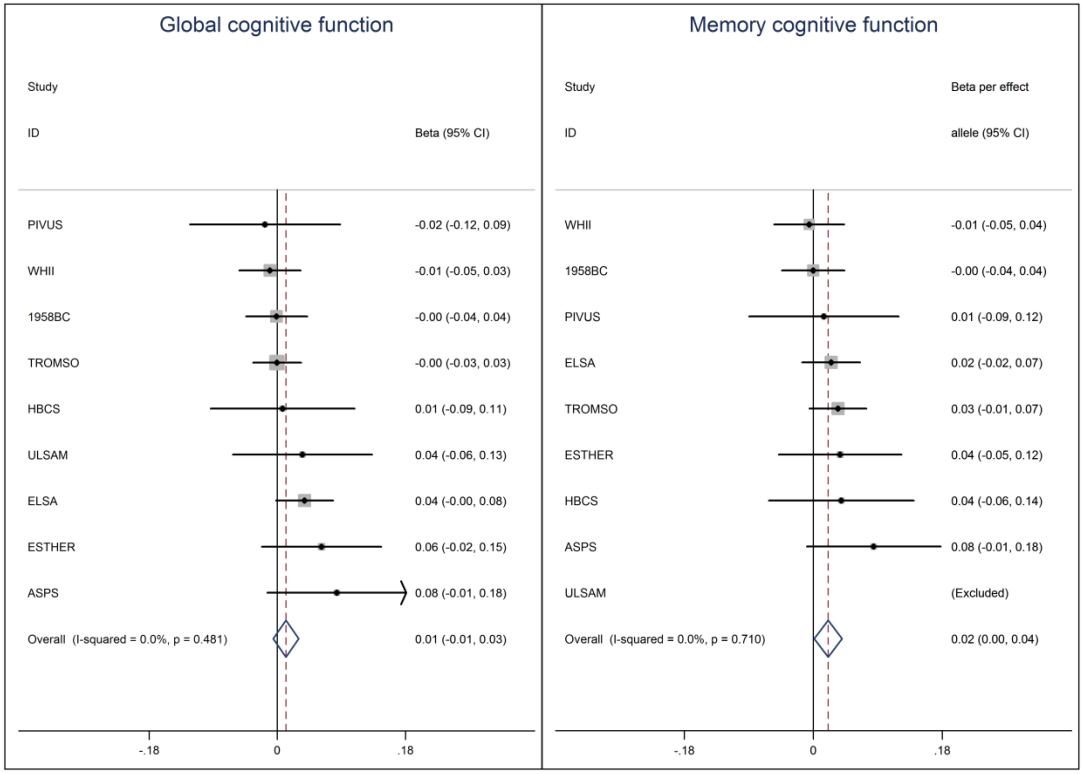


Figure 8.12: Association between *DHCR7* and cognitive function

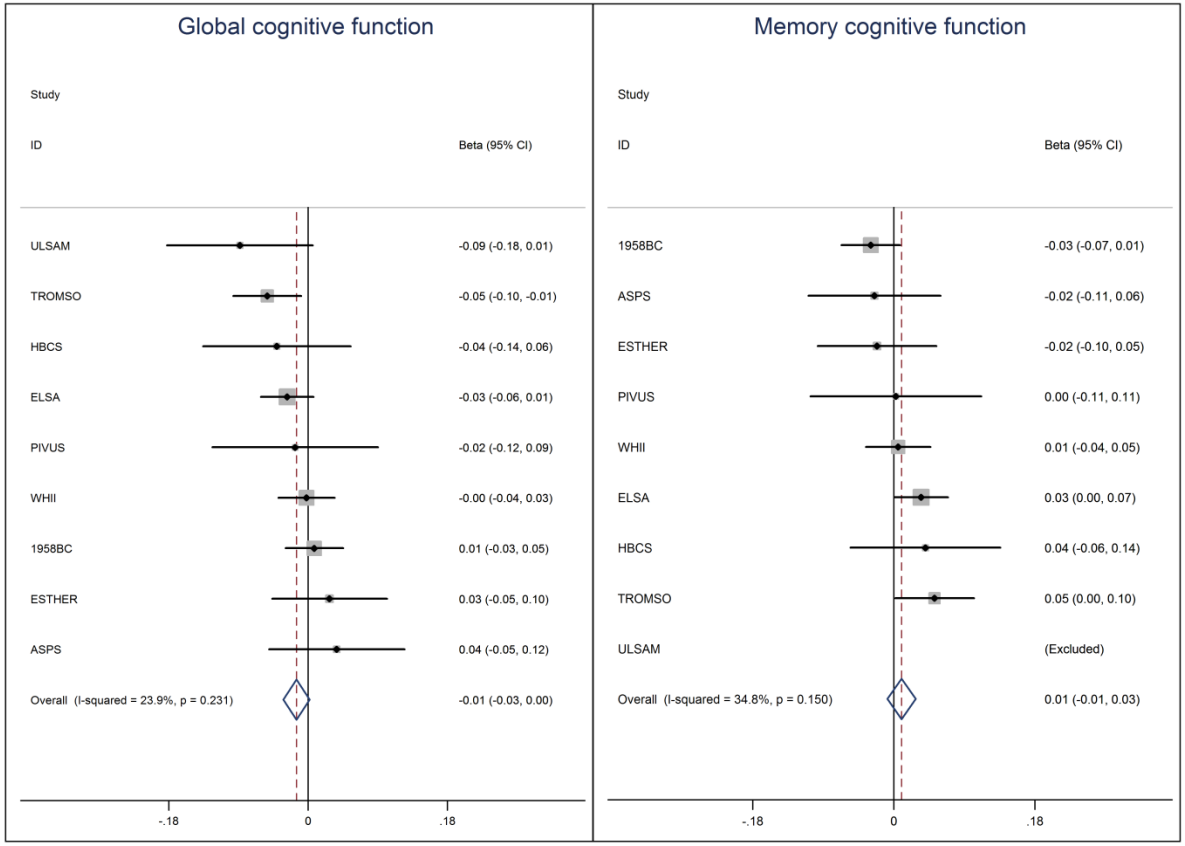


Figure 8.13: Association between *CYP2R1* and cognitive function

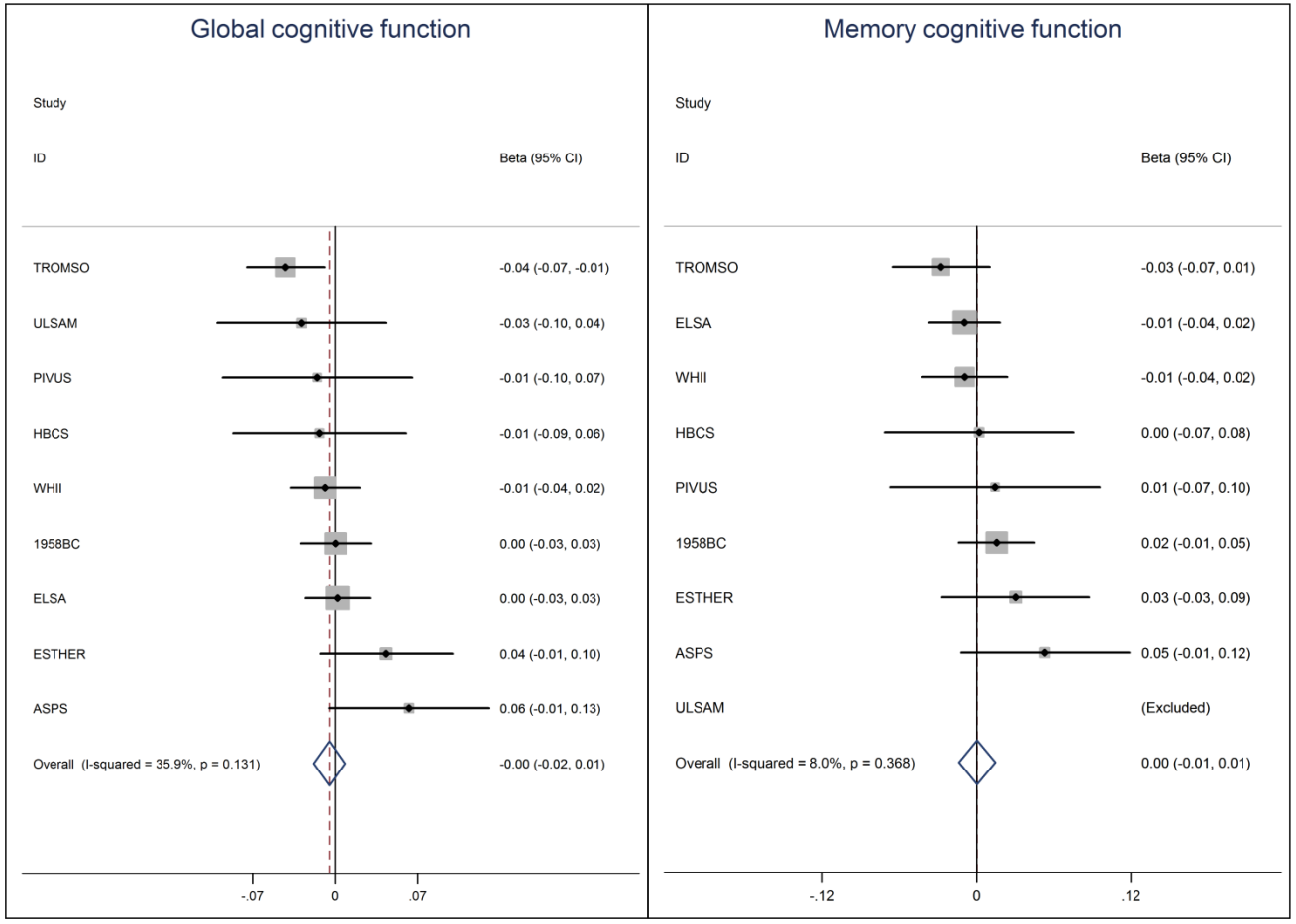


Figure 8.14: Association between synthesis score and cognitive function

Table 8.6: Meta-analysed association between genetic variants and cognitive function: Stratified by 25(OH)D tertiles

		Global Cognition				Memory cognition			
	25(OH)D tertiles	Coef	95% CI	Pvalue	$p_{heterogeneity}$	Coef	95% CI	Pvalue	$p_{heterogeneity}$
DHCR7	T1	0.002	(-0.04 to 0.05)	0.94	0.90	0.02	(-0.03 to 0.06)	0.49	0.74
	T2	0.02	(-0.02 to 0.06)	0.40	0.66	0.03	(-0.02 to 0.08)	0.18	0.60
	T3	0.01	(-0.03 to 0.05)	0.68	0.64	0.01	(-0.03 to 0.06)	0.63	0.58
CYP2R1	T1	-0.01	(-0.05 to 0.04)	0.83	0.18	0.02	(-0.03 to 0.07)	0.37	0.21
	T2	-0.05	(-0.09 to -0.01)	0.02	0.26	-0.03	(-0.09 to 0.02)	0.18	0.43
	T3	-0.02	(-0.06 to 0.03)	0.44	0.16	0.003	(-0.04 to 0.05)	0.91	0.21
Synthesis score	T1	-0.01	(-0.05 to 0.02)	0.46	0.57	0.01	(-0.03 to 0.05)	0.59	0.73
	T2	-0.01	(-0.05 to 0.02)	0.41	0.15	-0.002	(-0.04 to 0.03)	0.94	0.47
	T3	-0.01	(-0.05 to 0.05)	0.39	0.39	-0.01	(-0.04 to 0.03)	0.78	0.62

IV ratio

Calculation of the IV ratio is the final step in the MR analysis to estimate the causal effect of 25(OH)D on cognitive function (**Table 8.7**). There was no causal association between 25(OH)D and cognitive function using *DHCR7*, *CYP2R1* or the combined synthesis score.

In keeping with previous analyses, **Table 8.8** illustrates the IV ratio stratified by 25(OH)D tertiles. There was no causal association between 25(OH)D and cognitive function across any 25(OH)D tertiles and the estimates were inconsistent across the different genetic variants.

Meta-regression

In observational analyses, the association of 25(OH)D with global or memory cognitive function did not vary by the examined the study level factors of age group ($p_{meta\text{-}regression}$=0.97) and country ($p_{meta\text{-}regression}$=0.48).

Table 8.7: Instrumental variable ratio

	Genetic variant with 25(OH)D, per allele			Genetic variant with outcome, per allele			IV estimate for causal effect, per 10% increase in 25(OH)D		
	Coefficient, %	(se)	Pvalue	Coefficient*	(se)	P-value	Coefficient†	(se)	Pvalue
Global cognition									
DHCR7	2.28	(0.96)	0.02	0.01	(0.01)	0.19	0.05	(0.05)	0.25
CYP2R1	3.12	(0.43)	<0.001	-0.02	(0.01)	0.08	-0.05	(0.03)	0.09
Synthesis score	2.87	(0.33)	<0.001	-0.005	(0.01)	0.47	-0.02	(0.02)	0.47
Memory cognition									
DHCR7	2.28	(0.96)	0.02	0.02	(0.01)	0.04	0.09	(0.06)	0.12
CYP2R1	3.12	(0.43)	<0.001	-0.01	(0.01)	0.29	-0.03	(0.03)	0.30
Synthesis score	2.87	(0.33)	<0.001	0.0002	(0.01)	0.97	0.001	(0.03)	0.97

*Coefficient represents the difference in cognitive function (standard deviation(sd)) per vitamin D-increasing allele

† Calculated as the ratio between the genetic variant association with the outcome and 25(OH)D. Coefficients can be interpreted as change in cognitive function (sd) per 10% increase in 25(OH)D concentrations

Table 8.8: Instrumental variable ratio: Stratified by 25(OH)D tertiles

	Study-specific 25(OH)D tertiles	Genetic variant with 25(OH)D, per allele			Genetic variant with outcome, per allele			IV estimate for causal effect, per 10% increase in 25(OH)D		
		Coefficient	(se)	Pvalue	Coefficient	(se)	P-value	Coefficient	(se)	Pvalue
Global cognition										
DHCR7	T1	1.47	(0.54)	0.01	0.002	(0.02)	0.94	0.01	(0.15)	0.94
	T2	-0.17	(0.44)	0.70	0.02	(0.02)	0.40	-1.03	(2.96)	0.73
	T3	1.54	(0.39)	<0.001	0.009	(0.02)	0.02	0.06	(0.14)	0.68
CYP2R1	T1	-0.46	(0.50)	0.36	-0.006	(0.02)	0.80	0.13	(0.52)	0.81
	T2	0.07	(0.20)	0.71	-0.05	(0.02)	0.02	-6.54	(17.82)	0.71
	T3	2.96	(0.68)	<0.001	-0.02	(0.02)	0.44	-0.06	(0.08)	0.44
Synthesis score	T1	0.41	(0.38)	0.28	-0.01	(0.02)	0.46	-0.32	(0.52)	0.54
	T2	0.01	(0.16)	0.95	-0.01	(0.02)	0.41	-14.79	(247.8)	0.95
	T3	2.28	(0.28)	<0.001	-0.01	(0.02)	0.39	-0.06	(0.07)	0.39
Memory cognition										
DHCR7	T1	1.47	(0.54)	0.006	0.02	(0.02)	0.49	0.11	(0.17)	0.67
	T2	-0.17	(0.44)	0.70	0.03	(0.02)	0.18	-1.89	(5.12)	0.37
	T3	1.54	(0.39)	<0.001	0.01	(0.02)	0.63	0.07	(0.15)	0.48
CYP2R1	T1	-0.46	(0.50)	0.36	0.02	(0.02)	0.37	-0.47	(0.74)	0.52
	T2	0.07	(0.20)	0.71	-0.03	(0.02)	0.18	-4.21	(11.76)	0.72
	T3	2.96	(0.68)	<0.001	0.003	(0.02)	0.91	0.01	(0.08)	0.91
Synthesis score	T1	0.01	(0.02)	0.59	0.41	(0.38)	0.28	0.24	(0.51)	0.63
	T2	-0.002	(0.02)	0.94	0.01	(0.16)	0.95	-1.58	(33.11)	0.96
	T3	-0.002	(0.02)	0.78	2.28	(0.28)	<0.001	-0.02	(0.08)	0.78

8.4 Discussion

Results using data from nine European cohorts found that the non-linear observational association of 25(OH)D with both global and memory cognitive function replicates results from previous chapters. Both global and memory cognitive scores tended to be higher with increasing 25(OH)D concentrations amongst participants in the lower and mid-25(OH)D tertiles, while in the highest tertile, cognitive scores were lower with increasing 25(OH)D concentrations.

The genetic variants, *DHCR7*, *CYP2R1* and their combined synthesis score were associated with higher 25(OH)D concentrations. Therefore, it is expected that the genetic variants should be associated with higher global and cognitive function scores if there is a causal association between 25(OH)D and cognitive function. However, results indicate that the association with global or memory cognition was in the opposing direction for *DHCR7* and *CYP2R1*, and no overall association was observed for the synthesis score. Furthermore, when stratified by 25(OH)D tertiles, the relationships between the genetic variants and cognitive function did not mimic those evident in the observational analyses. The IV ratio, which was calculated overall and per tertile, reflected these inconsistent findings, implying that there is no evidence for 25(OH)D concentrations acting as a causal factor for cognitive function, when using *DHCR7* and *CYP2R1* as IVs.

Although MR analyses can contribute evidence when establishing a causal association between 25(OH)D and cognitive function, the gold standard method is an RCT. A limited number of RCTs have been conducted to assess the causal relationship between 25(OH)D and cognitive function (**Table 6.1**). In contrast to the MR findings, one RCT conducted amongst older adults (≥65years) based in the community found a significant difference in the treatment versus placebo groups over a period of one year (395). However, the treatment consisted of 4.0*μ*g of vitamin D in combination with vitamin A, β-carotene, thiamine, riboflavin, niacin, vitamin B_6, vitamin E, iron, zinc, copper, selenium, iodine, calcium and magnesium. Another RCT was consistent with MR results as there was no difference in cognitive tests amongst institutionalised older adults (≥65years who had evidence of mild-to moderate

cognitive impairment (Mini-mental State Examination score ≥10)) over 24 weeks (394). The treatment in this case involved a nutrient dense drink consisting of macronutrients, B-vitamin D, vitamin A, Vitamin D, vitamin E, vitamin K and vitamin C, minerals and trace elements. MR findings were supportive of results from the biggest RCT to date which was carried out amongst older women (≥65 years) who participated in the Women's Health Initiative (393). In this RCT, supplementation with 400 International Units (IU) of vitamin D and calcium over a mean follow-up of 7.8 years did not protect against cognitive impairment. Comparison between the MR study and these RCTs can be difficult as RCTs have mainly focused on older populations and vitamin D has been used in varying amounts and in combination with other nutrients.

Results from the meta-analysis indicate a strong non-linear relationship, suggesting that both lower and higher 25(OH)D concentrations may reduce cognitive function. Therefore, the association between the genetic variants and cognitive function was stratified into tertiles. There was no consistent evidence of an association between genetic variants across tertiles. However, the range of 25(OH)D concentrations in the highest tertile was 89.8nmol/l compared with 13.7nmol/l in T2 and 26.4nmol/l in T1. These large ranges may have led to spurious findings. Stratification by 25(OH)D tertiles could have led to collider bias if the association of two variables (i.e. genetic variants and cognitive function), changes upon conditioning on a third variable (i.e. 25(OH)D), when this third variable is affected by the other two (442). Although analyses has included 43,954 participants across 9 European cohorts, the genetic analyses is likely to be underpowered to detect small causal effects operating at the extremes of the 25(OH)D distribution. Power is an issue in MR studies due to the large sample size required to detect the small effect of a genetic variant on an exposure. Although a power calculation was beyond the scope of this thesis, an illustrative power calculation was conducted on a hypothetical MR study of the association between 25(OH)D and blood pressure which indicated that 80,000 participants would be required (207).

Results from meta-regression found no evidence that the association of 25(OH)D with global or memory cognitive function varied by age group (i.e. <65 versus ≥65 years), however, this could be due to lack of studies examining the

effect amongst older age groups. There was also no evidence of country level difference, which could be explained by the use of a genetically homogenous Caucasian population.

The success of an MR study relies upon the ability of the genetic variant to accurately proxy the exposure of interest (3). The choice of suitable genetic variants for MR analyses on 25(OH)D is thwarted by the complex metabolic pathway of vitamin D. There are numerous genes involved in the enzymatic conversions before vitamin D becomes a biologically active substance, making the choice of a reliable genetic variant more difficult. However, one study conducted in 2012, which evaluated genetic makers identified from genome wide association studies as instruments for MR studies on vitamin D found that *DHCR7* and *CYP2R1* were suitable instruments (207). Furthermore, the F-statistic, which takes into account the amount of variation in 25(OH)D due to the genetic variant, suggested that *DHCR7* and *CYP2R1* were considered strong instruments to proxy 25(OH)D (3).

The assumption of an independent association between the genetic variants and 25(OH)D (**Chapter 3**) was examined in the 1958BC. While the relationship between *CYP2R1* and 25(OH)D was not affected by adjustment for potentially important covariates, *DHCR7* was. Investigation of a direct association between *DHCR7* and these covariates revealed a significant association with region and use of suncover. This could imply that population stratification (**Chapter 3**) and, to some extent, behavioural factors may confound the relationship between *DHCR7* and 25(OH)D. These findings reduce the reliability of the role of *DHCR7* as an appropriate instrument to estimate the causal effect of 25(OH)D on cognitive function.

In addition to the independent association between genetic variants and 25(OH)D, the association should also be linear and unaffected by statistical interactions (3). The was no evidence of effect modification by covariates on the association between the genetic variants and 25(OH)D. While the association between *DHCR7* and 25(OH)D was linear, there was suggestion of a non-linear relationship between *CYP2R1* and 25(OH)D. When stratified by

tertiles, results implied the effect of *CYP2R1* on increasing 25(OH)D may be more prominent amongst participants with higher 25(OH)D concentrations.

Pleiotropy can also reduce reliability of the genetic variants to estimate 25(OH)D concentrations. Pleiotropy occurs when the genetic variant influence more than one phenotypic trait. It is not possible to formally test for the presence of pleiotropy (see **Chapter 3** for details) (443). An earlier study in 1958BC did not find evidence of pleiotropy following investigations of markers for cardiovascular health (207). However, the extent of potential effects of these genetic variants remains unknown at this stage. For example, *DHCR7* could influence cognitive function via its effect on cholesterol metabolism.

Conclusion

There was no evidence for 25(OH)D concentrations acting as a causal factor for cognitive performance in mid- to later-life. However, the observational association between 25(OH)D and cognitive function was strongly non-linear, and our genetic analyses may have been underpowered to detect small causal effects operating at the extremes of 25(OH)D distribution.

8.5 Summary

- Previous chapters have suggested a non-linear observational association between 25(OH)D and cognitive function
- The aim of this chapter was to assess the causal association between 25(OH)D and cognitive function using a Mendelian randomisation approach
- Data from nine European cohorts demonstrated a significant non-linear observational association between 25(OH)D and cognitive function, but there was no evidence of a genetic association
- Overall, there is uncertainty regarding a true causal non-linear association between 25(OH)D and cognitive function

Chapter 9 Discussion

Concern regarding the widespread global prevalence of low vitamin D status is amplified by the potential role of vitamin D in a variety of non-skeletal health outcomes, including brain function (56). Correspondingly, evidence for the role of vitamin D in both mental health and cognitive function has been accumulating. Since both mental health and cognitive function are of significant public health importance (**Chapter 1**), this thesis examined the two outcomes, with an emphasis on cognitive function.

The overall aim was to expand previous research by using a variety of study designs, including observational and genetic studies, to investigate whether vitamin D status (as measured by 25-hydroxyvitamin D, 25(OH)D) affects common mental disorders (CMD) and cognitive function. Observational findings were supportive of a non-linear association of 25(OH)D with CMDs and memory function. There was some suggestion that presence of apolipoprotein (*APOE*) ε4 alleles can modify the association between 25(OH)D and memory function. However, there was no evidence for a casual association between 25(OH)D and cognitive function using a Mendelian randomisation (MR) approach.

Mental health and cognitive function are complex entities, each influenced by an array of social, environmental, biological, psychological and genetic risk factors (**Chapter 1**) that can interact and accumulate over the life-course. Their complexity makes identification of explicit risk factors challenging. Moreover, there may be a certain period over the life-course during which specific risk factors manifest their importance. It is plausible that vitamin D can affect mental health and cognitive function throughout the life-course (**Chapter 1**). However, given the multitude of factors that can influence an individual's cognitive function and mental health, it is not surprising that the magnitude of the effect of 25(OH)D on both CMDs and cognitive function from observational study was small in comparison to, for example, the effects of age on cognitive function (101) or early life psychological health on CMDs (69).

This work focused on CMDs and cognitive function in adulthood, with an emphasis on mid-life (~45-50 years). CMDs, which are a leading cause of

Disability Adjusted Life Years (60), have been found to be particularly prevalent amongst adults in mid-life (9, 61). Furthermore, by examining cognitive function in mid-adulthood, there is potential to identify associations at a life stage when cognitive decline is only beginning to emerge.

9.1 Methodological considerations

Participants

Participants came primarily from the 1958 British birth cohort (1958BC). A major strength of this cohort is its large sample with extensive information on lifestyle, health and economic factors which have been gathered throughout the follow-up studies. The variables measured in each sweep of the 1958BC can be taken into account during analyses, which facilitates unravelling complex associations of vitamin D with CMDs and cognitive function. However, for some variables, such as vitamin D-related lifestyles, there was a reliance on self-reported data, which may have introduced some social desirability bias or random error. Furthermore there was no substantial information on dietary intake which limited the scope of the analyses.

Overall, cohort members of 1958BC are predominantly white, which although reflective of society at the time, is not representative of the demographic today (444). The 1958BC has been shown to represent the national population in terms of socioeconomic characteristics (233), marriage status and employment (216), however, it under-represents ethnic minorities (216). Additionally, like all longitudinal cohort studies, some sample attrition occurred in 1958BC where those with externalising or internalising behaviours and poor reading or maths scores were moderately under-represented (216). Despite this, participants remaining in the study at 45 years were representative of those in the original cohort (216) and results using inverse probability weights applied in **Chapters 5** and **6** did not differ from analyses without these weights.

Data from the eight additional European cohorts in **Chapter 8** enabled comparison to identify potential cohort effects. However, all data from these cohorts were from individuals with European ancestry, again reducing generalisability of findings to ethnic minorities.

Exposure: 25(OH)D

Vitamin D status was assessed using 25(OH)D concentrations. While this is not the biologically active form of vitamin D (**Figure 1.1**), it provides a useful marker of vitamin D exposure as it incorporates both endogenous synthesis and dietary intake (44). There have been some concerns about inaccurate measurements of serum 25(OH)D concentrations due to assay imprecision, however this has been partly overcome by calibration tools. 25(OH)D concentrations in the 1958BC were standardised to the mean of the Vitamin D External Quality Assessment Scheme (DEQAS) to enable comparison with other studies (235). Although DEQAS can provide standardised measures for 25(OH)D, there is still no guarantee that the 25(OH)D measurements are valid. Unfortunately, there was only one measure of 25(OH)D concentrations available from the 1958BC (at 45 years). While there is some evidence to suggest that 25(OH)D concentrations at a single time point are reflective of long-term status (445), it is possible that participants changed their behaviour, and consequently their vitamin D status, during the follow-up period in **Chapters 5** and **6**. Therefore, any inferences about the association between chronic or concurrent hypovitaminosis D and CMDs or cognitive function in observational study should be made with caution.

The cohort studies ESTHER, HBCS, PIVUS Tromsø and ULSAM used in **Chapter 8**, (with PIVUS also used in **Chapter 7**) applied different methods to measure 25(OH)D concentrations, which could add to the source of heterogeneity between studies.

Outcome: Mental Health

The 1958BC used standardised methods, which aim to minimise interviewer bias (239), to measure symptoms of CMDs. The Clinical-Interval Schedule Revised (CIS-R) and Mental Health-Inventory 5 (MHI-5) have been used in previous studies (**Chapter 4**). One advantage of the CIS-R is its ability to capture a variety of CMDs, such as depressive, anxiety, panic and phobia symptoms. This enabled investigation of the association between 25(OH)D with symptoms of different types of CMDs, which may have distinct biological mechanisms.

While these instruments can capture symptoms and signs of CMDs, they were not used as a clinical diagnostic assessment. Furthermore, the CIS-R and MHI-5 only capture symptoms over a short period of time (one week for CIS-R versus one month for MHI-5). This could introduce some bias since individuals with long-duration of symptoms may be over-represented and those with symptoms lasting only short periods may not be captured. The nuances between CIS-R and MHI-5 may have affected the ability to control for baseline CMDs in the prospective analyses of **Chapter 5**. Bias may have been introduced due to the potential reluctance of some individuals to respond to questions regarding mental health. Moreover, personality differences in people's perception of well-being may have influenced the reporting of symptoms. For example, it has been found that women who were more socially outgoing reported higher well-being on all dimensions (446).

Outcome: Cognitive function

Cognitive tests from the 1958BC and additional cohorts used in **Chapters 7** and **8** have been validated in previous studies (**Chapter 4**). In 1958BC, the influence of childhood cognitive ability on the subsequent association between 25(OH)D concentrations and cognitive function (**Chapter 6**) may have been underestimated due to different measures of cognitive function in childhood and adulthood.

A composite measure of global cognitive function was used in **Chapter 8** to obtain a more stable representation of cognitive function in the cohorts. Since previous studies have emphasised the role of vitamin D in Alzheimer's disease, a separate memory function score was created. Summed standardised scores from individual testes were used to create these scores. While factor analysis and principal components would be more complex alternatives to create a combined cognitive score, they were beyond the scope of this thesis.

Genetic data

Genotyping was subjected to specific quality control procedures as outlined in **Chapter 4**.

As discussed in **Chapters 3** and **8**, the success of a MR study is dependent on the ability of genetic variants to proxy 25(OH)D concentrations, be independent of confounding, and have no pleiotropic effects that may influence the outcome. The genetic variants all had an F-statistic >10, indicating their strength for use in MR study (221). There was some doubt on the role of *DHCR7* as it was found to be associated with region and use of suncover. However *DHCR7*, was robust to these assumptions when combined into a synthesis score along with *CYP2R1*. Nevertheless, the possibility of pleiotropic effects of either the individual genetic variants or synthesis score cannot be ruled out.

Statistical methods

Strengths and limitations of observational and genetic studies were described in detail in **Chapter 3**. While every effort was made to control for potential confounders in observational studies, the presence of unknown confounding cannot be ruled out. Furthermore, the presence of interaction, for example, by vascular pathologies on cognitive function, could be an important effect modifier in the relationship between 25(OH)D and outcomes. However, the exploration of all these possibilities was beyond the scope of this work.

A main limitation of the thesis was the lack of genetic study on CMDs. It was decided to use cognitive function as an example of genetic epidemiology to explore causal associations with 25(OH)D. This decision was partly due to a lack of validated statistical methods for binary outcomes for use in MR study (206).

There was a consistent non-linear association of 25(OH)D with CMDs and cognitive function. However, the studies, in particular genetic studies, may have been underpowered to detect small effects operating at the extremes of the 25(OH)D distribution. Furthermore, the potential of a non-linear association between 25(OH)D and cognitive function may affect the linearity assumption in MR analyses and its ability to estimate the causal effect (206, 443).

To determine the sample size required for MR analyses illustrative power calculations would have been required (207), but was beyond the scope of this thesis.

9.2 Main findings

1) Association of 25(OH)D with CMDs

There was evidence to support an association of low 25(OH)D concentrations with current and subsequent risk of depressive symptoms in mid-adulthood. There was some suggestion of a non-linear prospective relationship between 25(OH)D and depressive symptoms. Additionally, differences in vitamin D-related behaviours amongst those with symptoms of CMDs compared with those without CMDs, did not explain associations observed for low 25(OH)D concentrations. While a seasonal trend for CMDS was not observed, the extent to which season, rather than 25(OH)D status alone, affects CMDs remains uncertain. Interestingly, there was evidence of a cross-sectional association of 25(OH)D with depressive and panic symptoms but not with anxiety or phobia symptoms. These distinct relationships may reflect a true aetiological difference between CMDs, or may be an artefact of methodological limitations. One limitation of this study was lack of exploration of early life factors, such as emotional problems in the analyses, which reduced the ability to integrate a life-course perspective in the study.

Overall, this study strengthened the hypothesis of an association between 25(OH)D and CMDs in mid-life by providing insight into behavioural changes and examining a prospective approach.

2) Association of 25(OH)D with cognitive function

There was observational evidence of a non-linear association between 25(OH)D and a measure of memory function in mid-life. The novel approach in this study was the examination of cognitive function in a mid-adulthood (i.e. 50 years) sample. This study was supportive of previous work (**table 6.1**), and

went one step further to incorporate early life variables (i.e. educational attainment and childhood cognitive ability). Some of these early life variables may influence cognitive reserve, where the brain buffers the effects of neuropathology (447). Although these early life variable did attenuate the non-linear association between 25(OH)D and memory function, they did not fully explain it.

Further exploration identified that increasing 25(OH)D may be particularly beneficial for those with *APOE* ε4 alleles. The study was strengthened by the examination of a younger (50 years) and older (70 years) cohort. This adds to the complexity of the relationship between 25(OH)D and memory, implying that there are genetic factors which can mediate an individual's memory function.

However, there was no evidence for 25(OH)D concentrations acting as a causal factor for cognitive performance in mid- to later-life using methods of genetic epidemiology. This finding is in stark contrast with observational results. The interpretation of the study is complicated by the potential of a non-linear effect, therefore the lack of casualty identified could be due to methodological limitations. However, it is plausible that the low 25(OH)D concentrations observed are a consequence of having lower memory function.

9.3 Implications of findings

Findings support the hypothesis for an association between 25(OH)D and the brain. However, whether vitamin D can cause neurological changes, affecting CMDs and cognitive function remains uncertain. Evidence for a non-linear association was ubiquitous. There has been debate about whether a threshold for 25(OH)D concentrations exists for diseases (448-451), with further studies required to reach consensus. The threshold debate is important and it may be the basis for estimating vitamin D intake recommendations, and inform decisions of dosing amounts for RCTs. Findings from observational analyses demonstrate the threshold effect between 25(OH)D and depressive symptoms as well as 25(OH)D and memory cognitive function. Although, genetic studies indicate a deviation from a linear trend between 25(OH)D and cognitive

function, demonstrating a threshold effect was difficult due to the lack of participants in the extremes of the 25(OH)D distribution.

An interesting finding from this work was the non-linear association for 25(OH)D concentrations with both CMDs and cognitive function. It is possible that CMDs and cognitive function co-occur (**Chapter 1**). Interestingly, the association between 25(OH)D and cognitive function remained following adjustment for depressive symptoms.

Studies focused on mid-adulthood associations, and it is likely that the relationship of 25(OH)D with CMDs and in particular, with cognitive function will change with age. Previous studies have found that healthy dietary choice and physical activity in early mid-life may be beneficial in reducing the rate of cognitive decline and, ultimately, the risk of dementia (452). Therefore, findings presented in the thesis may provide some insight into potential interventions at a time point before the effects of neuropathology become apparent.

Evidence for effect modification by *APOE* ε4 may not have a clinical role due to ethical issues surrounding the disclosure of *APOE* genotyping results (453). However the gene-environment results presented raised potentially interesting mechanisms through which 25(OH)D may affect cognitive function that should be examined in future studies.

Overall, this work aspired to investigate potential mechanisms through which quality of life could be improved. The identification of an association between 25(OH)D and symptoms of CMDs, could support the development of cheap effective public health policies to prevent or improve symptoms of CMDs which are often left undiagnosed and untreated throughout an individual's life. Similarly, avoiding low 25(OH)D concentrations could delay the onset of cognitive decline in later life, and improve the quality of life of individuals (154). Vitamin D status tends to decrease with increasing age (**Chapter 1**). Adults who are deficient in vitamin D have a higher risk of osteoporosis, muscle weakness, fractures and falls which could affect their physical function and their ability to partake in social interactions, thereby increasing their risk of CMDs and cognitive decline. Therefore, although evidence for a causal relationship of

25(OH)D with CMDs and cognitive function was lacking, ensuring public health messages to avoid vitamin D deficiency amongst adults is justified.

9.4 Potential areas for future research

In order to validate findings, analyses conducted during this thesis would require replication, preferably in larger cohorts. Examining the association between 25(OH)D and cognitive function in mid-life provides a bases for future studies. It would be interesting to see how the associations will change as the 1958BC ages and cognitive decline and dementia become evident. Furthermore, larger meta-analyses that incorporate a variety of ages could provide some insight into the age differences in the associations.

It would be beneficial to examine the associations of 25(OH)D with CMDs and cognitive function amongst ethnic minorities to get a broader view of the situation amongst the general population, and amongst specific population sub-groups. Ideally in future studies, 25(OH)D would be measured at various points over the life-course and both CMDs and cognitive function would consist of comparable, repeated measures. These methods would allow investigation of a life-course association, which would be beneficial to the design of future public health interventions.

As statistical methods for MR studies are developed, it would be interesting to investigate the causal role of 25(OH)D in CMDs. The gold standard approach would be large, long-term RCT which could use various doses of 25(OH)D with participants with varying baseline 25(OH)D concentrations to determine the if there is a causal role of 25(OH)D in CMDs and cognitive function. This method would allow for exploration of the most effective dose and time point to target an intervention for prevention of mental illness and reduced cognitive function.

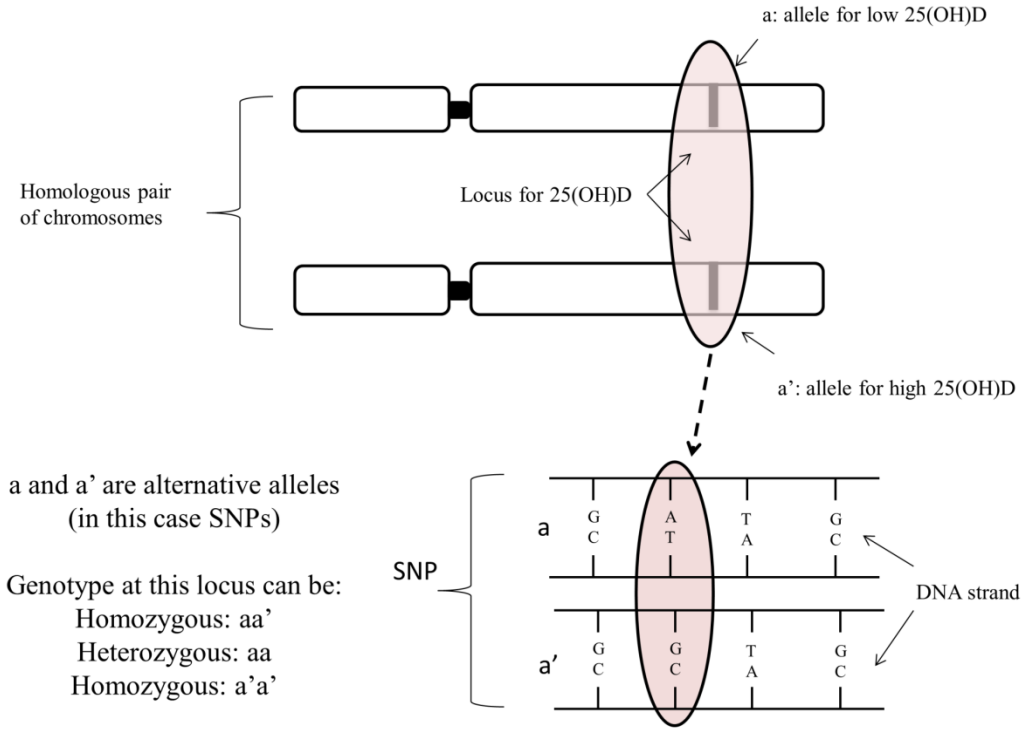
Appendix 2 Additional information for Chapter 3

Appendix 2.1: Glossary of genetic terms (3, 187):

- *Alleles* are variant forms of a single-nucleotide polymorphism (SNP), a specific polymorphic site or a whole gene detectable at a locus
- A *Chromosome* carries a collection of genes located on a string of DNA. A homologous chromosome carries the same collection of genes, but each gene can be represented by a different allele on the two homologues. Offspring will receive one of those homologues, but not both
- Calculating the *call rate* will give the amount of missing genotypes of each SNP or individual. Low call rates indicate poor genotyping quality.
- *Discordance* refers to the presence of a specific trait in only one member of a pair of for example, identical twins
- *DNA* (deoxyribonucleic acid) is a molecule containing genetic instructions used in development and function of organisms. DNA consists of sequence containing four nucleotide bases, Adenine (A), Tyrosine (T), Guanine (G) and Cytosine (C). Two strands of DNA form a double-helix in which A on one strand bind with T on the other as G binds with C
- A *gene* is a unit of inheritance consisting of a DNA sequence, including introns, exons and regulatory regions
- *Genotype* is the genetic makeup of an individual. It refers to the two alleles inherited at a specific locus. If they are the same, the genotype is homozygous, if they are different, heterozygous
- *Genotyping* is the process of determining which genetic variants an individual possesses
- *Haplotype* is the set of alleles present at a series of linked loci on a chromosome; a person has two haplotypes for any such series of loci, one inherited from the mother and the other from the father
- Under *Hardy-Weinberg equilibrium* (HWE), alleles segregate randomly in the population, allowing expected genotype frequencies to be calculated from allele frequencies. For example, if the allele G is p and T is (1- p), the expected frequencies of genotypes GG, GT and TT are:

p2+ 2pq+q2 =1. Comparison of expected versus observed genotype frequencies provides a test of HWE using a chi-square statistic

- *Heterozygosity* refers to having different alleles at one or more corresponding chromosomal loci
- A *locus* is the position in a DNA sequence and can be used to refer to a SNP or to a larger region of DNA sequence
- *Minor allele frequency* (MAF) is the frequency at which the least common allele occurs in a given population
- *Meiosis* is a type of cell division necessary for reproduction
- *Nucleotides* are molecules that serve as subunits of nucleic acids like DNA
- Polymorphism is the existence of two or more variants at a locus. It is usually restricted to common genetic variants i.e. above 1% prevalence in the population. Below this, variants are referred to as mutations
- Single nucleotide polymorphisms (SNPs) are positions along a chromosome where the genetic code varies between individuals by a single base pair


a: allele for low 25(OH)D
Homologous pair of chromosomes
Locus for 25(OH)D
a': allele for high 25(OH)D
a and a' are alternative alleles (in this case SNPs)
Genotype at this locus can be:
Homozygous: aa'
Heterozygous: aa
Homozygous: a'a'
SNP
a
a'
G C
A T
T A
G C
G C
G C
T A
G C
DNA strand

Appendix 3 Additional information for Chapter 5

Appendix 3.1: PubMed search terms

((Depression OR Major Depressive Disorder OR Affective Mood disorders OR Mood disorder OR Dysthymia OR Dysthymic disorder OR Anxiety OR generalized anxiety disorder OR Panic OR Phobia OR Common mental disorders OR Psychiatric disorders)) AND ("Vitamin D"[MeSH Terms] OR cholecalciferol OR ergocalciferol OR "vitamin D" OR "vitamin D3" OR "vitamin D2" OR "Vitamin D"[Substance] OR "vitamin D deficiency"[mesh] OR Calcifediol [mesh] OR "25-hydroxyvitamin D"[text word] OR "25(OH)D"[text word]).

Appendix 3.2: Results from systematic review

Author (year), country	Setting (Study Name)	Groups	Adjustment
Cross-sectional			
Kwasky (2012)(309), US	University campus	African American Caucasian	none
Menkes (2012) (310), New Zealand	Psychiatric inpatients	Schizophrenia (n=38); Schizoaffective (n=11); Bipolar disorder (n=19); Depression (n=17); Miscellaneous (n=17)	none
Zhao (2010) (311), US	NHANES		age, gender, race/ethnicity, education, marital status, BMI, cotinine(smoking), physical activity, alcohol, creatinine, chronic conditions
Nanri (2009) (312), Japan	Municipal office employees		workplace, age, gender, BMI, marital status, job position, occupation, non-job physical activity, smoking, alcohol consumption and folate
Armstrong (2007)(313)		Fibromyalgia	none
Jaddou (2012)(314), Jordan	Community		age, gender, marital status, education, BMI, serum creatinine, number of chronic diseases, smoking, exercise and altitude
Ganji (2010)(315), US	NHANES		age, gender, race/ethnicity, education, smoking, physical activity, alcohol drinking, BMI, abdominal obesity and serum calcium concentrations

Knippenberg (2010)(316), Netherlands	MS outpatients and patients		age expanded Disability Status Scale, fatigue
Kjærgaard (2011)(317), Norway	Tromsø	Community	age, gender, BMI, physical exercise, alcohol, education, marital status, kidney function and chronic disease
Hoang (2011)(318), US	CCLS		
Pan (2009)(319), China	NHAPC		age, gender, urban/rural, BMI, physical activity, smoking , social activity level, marital status, household income and number of chronic diseases, geographic location
Motsinger (2012)(320), US	IWHS	postmenopausal women	age, energy intake, BMI, education, smoking, living arrangement, antidepressant usage, comorbidity history and physical activity
Lee (2010)(321), Italy, Belgium, Poland, Sweden, UK, Spain, Hungary, Estonia	EMAS		age, physical activity, BMI, physical function, serum creatinine, number of morbidities, number of adverse life events
Stewart (2010)(322), UK	HSE		age, gender, social class, season of examination, physical health status
Hoogendijk (2008)(323), Netherlands	Longitudinal Ageing Study Amsterdam	Cases: Female subgroup (n=33) from psychiatric inpatients Controls: (n=691) no psychiatric illness from GOS	age, gender, smoking, BMI, chronic conditions, serum creatinine, season, urbanization and physical activity
Wilkins (2006)(324), US	Community	40 Mild dementia 40 non-demented	age, season, race, gender

Johnson (2008)(325), US	OAANP		age, gender, race

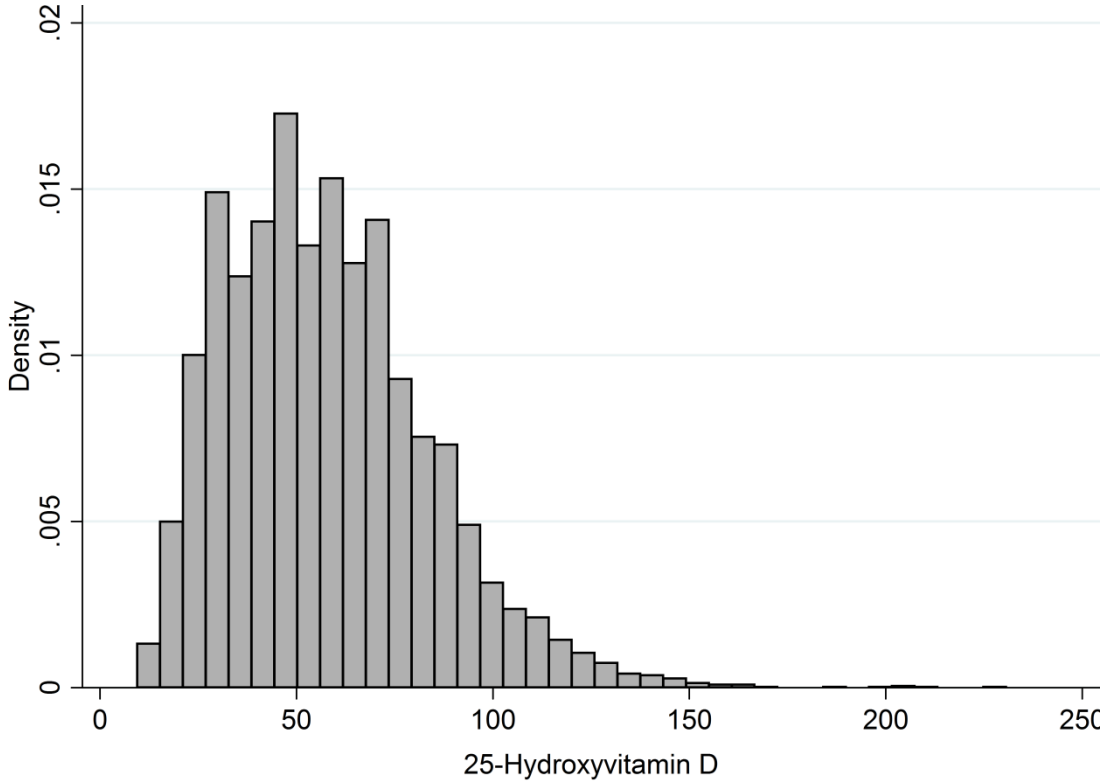
Case-control			
Cizza (2012)(326), Italy	POWER	premenopausal with MDD (n=92); premenopausal controls (n=44)	
Eskandari (2007)(327)	89 MDD 44 control		none
Schneider (2000)(328), Germany	out-patient clinic	34 schizophrenia 30 alcohol addiction 25 major depression 31 healthy controls	none
Oren (1994)(329), US	15 SAD 15 age and gender-matched controls		none
Michelson (1996) (330), US	24 past or current major depression 24 controls, age and BMI-matched		none
Berk (2008)(331), Australia	Psychiatric inpatients, with predominately mood, adjustment, personality and substance use disorders Bipolar (n=18) Depression (n=14) Personality disorder (n=5) Alcohol/substance (n=4) Post-traumatic stress (n=2) Anxiety (n=1 Schizoaffective (n=1)	Cases: Female subgroup (n=33) from psychiatric inpatients Controls: (n=691) no psychiatric illness from GOS	Age, season

	Unknown (n=9)		
Herrán (2000)(332), Spain	Patients in mental health unit	19 Depressive 19 Controls, age matched	none
Jorde (2006)(333), Norway	Community (Tromsø)	21 SHPT 63 Controls	none
Cohort			
Bertone-Johnson (2011)(334), US	WHI, 3 years	postmenopausal women	age, race/ethnicity, BMI, waist-to-hip ratio, education, smoking, alcohol, past hormone therapy use, totally energy intake, marine omege-3 fatty acid intake, marital status, physical activity, physical function score, history of cardiovascular disease and solar irradiance
Chan (2011)(335), China	Community, 4 year follow-up		age, number of diseases, smoking, alcohol BMI, physical activity, mobility limitations, dietary intake, season of blood measurement and PTH
Milaneschi (2010)(336), Italy	InCHIANTI, 6 year follow-up		Age, gender, education, smoking, alcohol,. MMSE score, BMI, season, number of drugs, vitamin D supplements, physical activity, chronic diseases
May (2010)(337), US	CVD patients, follow-up 1.07 ±1.13 years		PTH, season
Randomised-controlled trial			

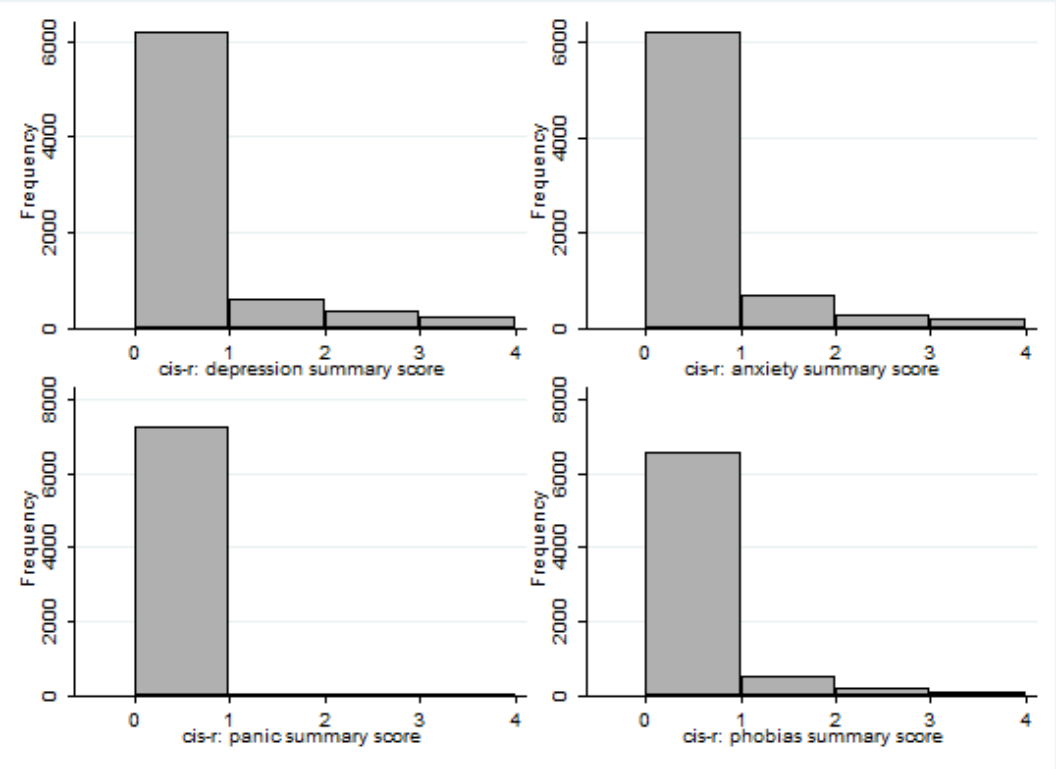
Lansdowne (1998)(338), Australia	University campus, 5 days	Treatment 1 n=150; 400IU/day and 9000IU vitamin A/day Treatment 2 n=142; 8000IU/day Control: n=149 0IU/day and 10,000IU vitamin A/day	none
Dean (2011)(339), Australia	Community, 6 weeks	Treatment n=63 5000IU/day Control: n=65 placebo/day	NA
Harris (1993)(340), US	community, 1 year	Treatment (n=125): 400 IU/day and 337mg calcium/day Control: (n=125): 0IU/day and 337mg calcium/day	none
Jorde (2008)(341), Norway	BMI 28-47kb/m2, 1 year	Treatment 1 n=150; 20,000 and 20,000IU/week Treatment 2 n=142; 20,000IU and placebo/week Control: n=149 2 placebo/week	Na
Bertone-Johnson (2011)(334), US	WHI, 2 year intervention	Treatment n=18,176 400IU/day with 1,000mg calcium Control: n=12,421 placebo/day	age, race, WHI hormone Trial intervention, WHI dietary Modification Trial intervention and depressive symptoms above the threshold level at year 1
Kjærgaard (2012)(342), Norway	Tromsø, 6-month	Treatment n=180 with 25(OH)D <55nmol/l; 2 x20 000IU/week Control: n=75 with 25(OH)D >70nmol/l; 2 placebo 000IU/week	NA

Sanders (2011)(343), Australia	Vital D study , 3-5 years	Treatment n=1018 500,000IU every autumn/winter Control: n=1016 placebo	
Before-after study			
Sanders (2011)(344), US	Patients being treated for vitamin D deficiency <40ng/mL, 8 week follow-up		na

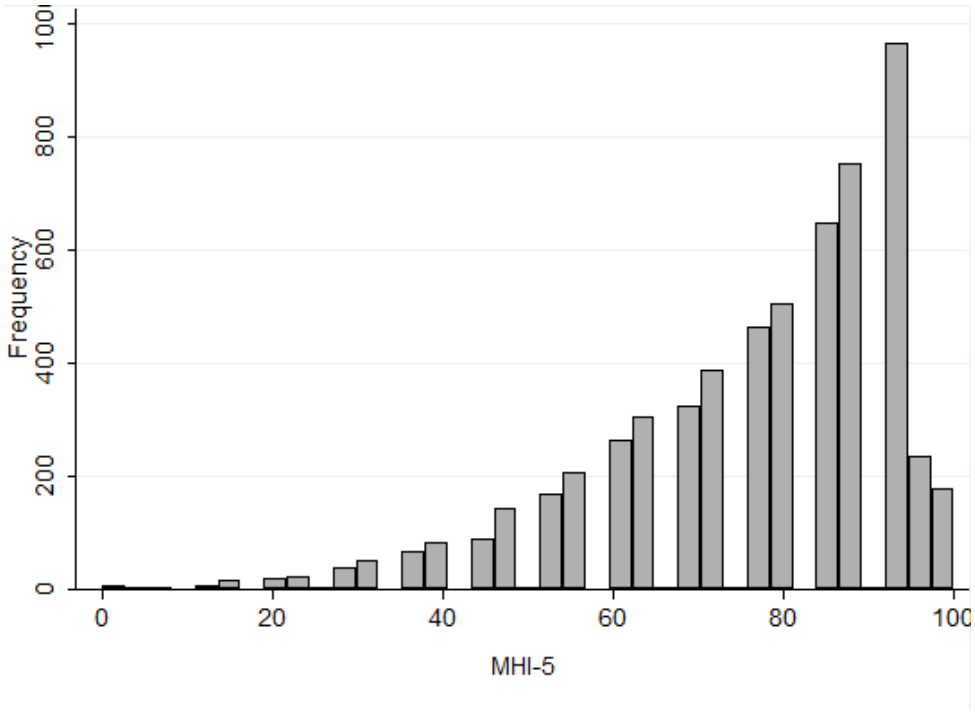
Appendix 3.3: Distribution of 25(OH)D in the sample



Appendix 3.4: Distribution of CMDS in the sample, before categorisation



Appendix 3.5: Distribution of MHI-5, before categorisation



Appendix 3.6: Geometric mean of 25(OH)D according to vitamin D-related lifestyles at 45 years

	Total*	Geometric mean 25(OH), nmol/l (95% CI)†
Time spent outside per day		
<3 hours	3,886	48.33 (47.64, 49.04)
≥3 hours	2,944	58.18 (57.24, 59.14)
Missing	571	49.50 (47.50, 51.57)
p‡		<0.001
TV/PC time per day		
<3 hours	4,695	54.66 (53.96, 55.37)
≥3 hours	2,376	48.09 (47.16, 49.03)
Missing	330	47.45 (44.87, 50.18)
p‡		<0.001
Use of sun cover		
most times	6,188	52.76 (52.16, 53.37)
rarely	666	48.08 (46.26, 49.98)
Missing	547	50.19 (48.13, 52.35)
p‡		<0.001
Blistering after sunburn		
rarely/never/sometimes	6,436	52.59 (51.99, 53.19)
often	129	41.99 (38.91, 45.32)
Missing	836	50.39 (48.72, 52.12)
p‡		<0.001
Seeking sun tan		
rarely/never/sometimes	5,254	49.76 (49.14, 50.39)
often	1391	63.96 (62.56, 65.40)
Missing	756	49.41 (47.70, 51.19)
p‡		<0.001
Vitamin D-containing supplements		
< daily	6,058	50.72 (50.11, 51.32)
≥ daily	1,185	60.80 (59.43, 62.20)
Missing	158	47.18 (43.54, 51.14)
p‡		<0.001
Oily fish consumption		
≥ weekly	2,191	54.49 (53.45, 55.55)
< weekly	5,052	51.32 (50.66, 51.99)
Missing	158	46.50 (42.84, 50.48)
p‡		<0.001
Margarine consumption		
≥ weekly	4,563	52.83 (52.14, 53.53)
< weekly	2,601	51.18 (50.23, 52.15)
Missing	237	49.27 (46.15, 52.61)
p‡	0.01	

*Based on eligible sample for Chapter 5 (N=7,401)

‡ Data obtained when participants were aged 45 years. 25(OH)D range: 9.5-230.7nmol/l

‡ P value from linear regression with naturally log-transformed 25(OH)D adjusted for gender and SEP in adulthood

Appendix 3.7: Association between 25(OH)D and CMDs: complete case results

	25- Hydroxyvitamin D, nmol/l					
	<25	25-49.9 OR (95% CI)	50-74.9 OR (95% CI)	75-99.9 OR (95% CI)	≥100 OR (95% CI)	P_{trend}
Depressive symptoms						
Model 1* (n=7401)	1.0	0.64 (0.48, 0.85)	0.50 (0.37, 0.67)	0.43 (0.31, 0.62)	0.32 (0.19, 0.52)	< 0.001
Model 2 (n=7401)	1.0	0.68 (0.52, 0.91)	0.55 (0.41, 0.74)	0.47 (0.33, 0.67)	0.34 (0.21, 0.57)	< 0.001
Model 3 (n=7376)	1.0	0.71 (0.53, 0.94)	0.58 (0.43, 0.79)	0.51 (0.35, 0.73)	0.37 (0.22, 0.62)	< 0.001
Model 4 (n=6132)	1.0	0.73 (0.52, 1.03)	0.67 (0.46, 0.96)	0.59 (0.38, 0.91)	0.40 (0.22, 0.74)	0.003
Anxiety symptoms						
Model 1* (n=7401)	1.0	0.80 (0.58, 1.12)	0.64 (0.45, 0.90)	0.67 (0.45, 0.98)	0.68 (0.42, 0.98)	0.03
Model 2 (n=7401)	1.0	0.83 (0.59, 1.16)	0.68 (0.48, 0.96)	0.70 (0.48, 1.04)	0.73 (0.45, 1.18)	0.07
Model 3 (n=7376)	1.0	0.82 (0.59, 1.15)	0.68 (0.48, 0.97)	0.72 (0.48, 1.07)	0.75 (0.46, 1.22)	0.12
Model 4 (n=6132)	1.0	0.97 (0.66, 1.43)	0.90 (0.60, 1.35)	1.00 (0.63, 1.59)	1.09 (0.62, 1.93)	0.83
Panic symptoms						
Model *1 (n=7401)	1.0	0.42 (0.24, 0.75)	0.38 (0.21, 0.68)	0.19 (0.08, 0.43)	0.20 (0.07, 0.62)	< 0.001
Model 2 (n=7401)	1.0	0.46 (0.26, 0.83)	0.44 (0.24, 0.81)	0.22 (0.09, 0.50)	0.24 (0.08, 0.73)	0.001
Model 3 (n=7376	1.0	0.48 (0.26, 0.87)	0.49 (0.26, 0.91)	0.25 (0.10, 0.58)	0.28 (0.09, 0.87)	0.004
Model 4 (n=6010)	1.0	0.41 (0.20, 0.83)	0.64 (0.31, 1.33)	0.27 (0.10, 0.79)	0.47 (0.13, 1.62)	0.20
Phobia symptoms						
Model 1* (n=7401)	1.0	0.62 (0.42, 0.92)	0.57 (0.38, 0.86)	0.52 (0.33, 0.84)	0.17 (0.07, 0.41)	< 0.001
Model 2 (n=7401)	1.0	0.66 (0.44, 0.98)	0.63 (0.41, 0.94)	0.56 (0.35, 0.90)	0.18 (0.07, 0.44)	< 0.001
Model 3 (n=7376	1.0	0.65 (0.44, 0.97)	0.63 (0.42, 0.96)	0.58 (0.36, 0.94)	0.19 (0.08, 0.46)	0.001
Model 4 (n=6132)	1.0	0.73 (0.46, 1.16)	0.80 (0.50, 1.30)	0.76 (0.44, 1.34)	0.28 (0.11, 0.71)	0.09

*Model 1 was adjusted for gender & season. Model 2 was adjusted for gender, season & socioeconomic position. Model 3 was adjusted for gender, season & socioeconomic position and Body Mass Index. Model 4 was adjusted for gender, season, socioeconomic position, Body Mass Index, smoking and physical activity, PC/TV leisure time, suncover, blistering after sunburn, actively seeking suntan.

Appendix 3.8: Association between 25(OH)D and CMDs: weighted-case results

	25- Hydroxyvitamin D, nmol/l					
	<25	25-49.9 OR (95% CI)	50-74.9 OR (95% CI)	75-99.9 OR (95% CI)	≥100 OR (95% CI)	P_{trend}
Depressive symptoms						
Model 1* (n=7154)	1.0	0.66 (0.49, 0.88)	0.50 (0.36, 0.68)	0.44 (0.30, 0.64)	0.33 (0.20, 0.56)	< 0.001
Model 2 (n=7154)	1.0	0.70 (0.52, 0.94)	0.55 (0.40, 0.75)	0.48 (0.33, 0.70)	0.36 (0.22, 0.61)	< 0.001
Model 3 (n=7132)	1.0	0.73 (0.54, 0.98)	0.59 (0.42, 0.81)	0.53 (0.36, 0.77)	0.40 (0.24, 0.68)	< 0.001
Model 4 (n=5935)	1.0	0.76 (0.53, 1.08)	0.69 (0.47, 1.00)	0.59 (0.38, 0.93)	0.43 (0.23, 0.80)	0.01
Anxiety symptoms						
Model 1* (n=7154)	1.0	0.79 (0.56, 1.11)	0.62 (0.43, 0.90)	0.64 (0.42, 0.96)	0.66 (0.40, 1.08)	0.03
Model 2 (n=7154)	1.0	0.82 (0.58, 1.16)	0.66 (0.46, 0.95)	0.68 (0.45, 1.03)	0.70 (0.43, 1.16)	0.06
Model 3 (n=7132)	1.0	0.81 (0.57, 1.14)	0.67 (0.46, 0.96)	0.69 (0.46, 1.05)	0.72 (0.43, 1.19)	0.10
Model 4 (n=5936)	1.0	0.97 (0.65, 1.44)	0.87 (0.57, 1.33)	0.94 (0.56, 1.54)	1.03 (0.58, 1.80)	0.90
Panic symptoms						
Model 1* (n=7154)	1.0	0.42 (0.23, 0.75)	0.36 (0.20, 0.66)	0.20 (0.08, 0.47)	0.19 (0.06, 0.59)	< 0.001
Model 2 (n=7154)	1.0	0.45 (0.25, 0.82)	0.41 (0.23, 0.75)	0.23 (0.10, 0.55)	0.22 (0.07, 0.68)	0.001
Model 3 (n=7132)	1.0	0.47 (0.25, 0.88)	0.46 (0.24, 0.88)	0.27 (0.10 0.67)	0.27 (0.08, 0.85)	0.01
Model 4 (n=5858)	1.0	0.39 (0.19, 0.83)	0.62 (0.30, 1.30)	0.26 (0.09, 0.76)	0.44 (0.13, 1.15)	0.16
Phobia symptoms						
Model 1* (n=7154)	1.0	0.67 (0.45, 1.01)	0.61 (0.40, 0.92)	0.53 (0.33. 0.86)	0.19 (0.08, 0.46)	< 0.001
Model 2 (n=7154)	1.0	0.71 (0.46, 1.07)	0.66 (0.43, 1.01)	0.57 (0.65, 0.92)	0.20 (0.08, 0.50)	< 0.001
Model 3 (n=7132)	1.0	0.70 (0.46, 1.06)	0.66 (0.43, 1.02)	0.56 (0.35, 0.95)	0.21 (0.08, 0.51)	0.001
Model 4 (n= 5817)	1.0	0.39 (0.19, 0.83)	0.62 (0.30, 1.30)	0.26 (0.09, 0.76)	0.44 (0.14, 1.46)	0.17

* Model 1 was adjusted for gender & season. Model 2 was adjusted for gender, season & socioeconomic position. Model 3 was adjusted for gender, season & socioeconomic position and Body Mass Index. Model 4 was adjusted for gender, season, socioeconomic position, Body Mass Index, smoking and physical activity, PC/TV leisure time, suncover, blistering after sunburn, actively seeking suntan.

Appendix 3.9: Association between 25(OH)D and depressive symptoms at 50 years: complete case results

	25- Hydroxyvitamin D, nmol/l						
	<25	25-49.9 OR (95% CI)	50-74.9 OR (95% CI)	75-99.9 OR (95% CI)	≥100 OR (95% CI)	P_{trend}	$P_{curvature}$
Depressive symptoms (50 years)							
Model 1* (n=5966)	1.0	0.71 (0.53, 0.94)	0.59 (0.43, 0.79)	0.58 (0.41, 0.81)	0.57 (0.37, 0.87)	0.002	< 0.001
Model 2 (n= 5966)	1.0	0.73 (0.54, 0.97)	0.61 (0.45, 0.82)	0.61 (0.43, 0.85)	0.60 (0.39, 0.92)	0.006	0.001
Model 3 (n= 5966)	1.0	0.78 (0.58, 1.05)	0.67 (0.49, 0.91)	0.68 (0.47, 0.96)	0.71 (0.46, 1.10)	0.049	0.001
Model 4 (n= 5947)	1.0	0.79 (0.58, 1.06)	0.70 (0.51, 0.95)	0.72 (0.51, 1.03)	0.78 (0.50, 1.21)	0.164	0.001
Model 5 (n=5095)	1.0	0.90 (0.64, 1.26)	0.88 (0.61, 1.25)	0.92 (0.61, 1.38)	1.13 (0.68, 1.85)	0.690	0.03

* Model 1 was adjusted for gender & season. Model 2 was adjusted for gender, season & socioeconomic position. Model 3 was adjusted for gender, season & socioeconomic position and presence of CMDS at 45 years. Model 4 was adjusted for gender, season, socioeconomic position presence of CMDS at 45 years and Body Mass Index, Model 5 was adjusted for gender, season, socioeconomic position presence of CMD at 45 years, BMI, smoking and physical activity, PC/TV leisure time, suncover, blistering after sunburn, actively seeking suntan.

Appendix 3.10: Association between 25(OH)D and depressive symptoms at 50 years: weighted-case results

	25- Hydroxyvitamin D, nmol/l						
	<25	25-49.9 OR (95% CI)	50-74.9 OR (95% CI)	75-99.9 OR (95% CI)	≥100 OR (95% CI)	P_{trend}	$P_{curvature}$
Depressive symptoms (50 years)							
Model 1* (n=5772)	1.0	0.74 (0.55, 0.99)	0.60 (0.44, 0.81)	0.55 (0.39, 0.78)	0.56 (0.36, 0.87)	0.001	0.001
Model 2 (n=5772)	1.0	0.76 (0.56, 1.02)	0.62 (0.46, 0.85)	0.58 (0.41, 0.83)	0.60 (0.38, 0.93)	0.003	0.001
Model 3 (n=5772)	1.0	0.81 (0.60, 1.09)	0.68 (0.50, 0.92)	0.64 (0.45, 0.91)	0.70 (0.45, 1.10)	0.020	0.001
Model 4 (n=5756)	1.0	0.81 (0.60, 1.10)	0.70 (0.52, 0.96)	0.68 (0.47, 1.00)	0.76 (0.48, 1.19)	0.072	0.001
Model 5 (n=4937)	1.0	0.90 (0.64, 1.27)	0.87 (0.61, 1.25)	0.85 (0.56, 1.28)	1.09 (0.66, 1.83)	0.994	0.02

N=5966b Model 1 was adjusted for gender & season. Model 2 was adjusted for gender, season & socioeconomic position. Model 3 was adjusted for gender, season & socioeconomic position and presence of CMDS at 45 years. Model 4 was adjusted for gender, season, socioeconomic position presence of CMD at 45 years and Body Mass Index, Model 5 was adjusted for gender, season, socioeconomic position presence of CMDS at 45 years, BMI, smoking and physical activity, PC/TV leisure time, suncover, blistering after sunburn, actively seeking suntan.

Appendix 3.11: Association between CMD and 25(OH)D where 25(OH)D is the outcome: complete case results

	N	Coefficient (95% CI), % change in 25(OH)D	P_{trend}
Depressive symptoms			
Model 1*	7,401	-10.15 (13.74, -6.56)	<0.0001
Model 2	7,194	-8.42 (-12.09, -4.75)	<0.0001
Model 3	7,171	-7.06 (-10.68, -3.44)	<0.0001
Model 4	5,966	-4.68 (-8.53, -0.83)	0.02
Anxiety symptoms			
Model 1*	7,401	-4.78 (-0.09, -8.66)	0.02
Model 2	7,194	-3.192 (-7.13, 0.74)	0.11
Model 3	7,171	-2.48 (-6.35, 1.39)	0.21
Model 4	5,966	0.77 (-3.25, 4.79)	0.71
Panic symptoms			
Model 1*	7,401	-19.71 (-27.91, -11.52)	<0.0001
Model 2	7,194	-17.72 (-26.17, -9.28)	<0.0001
Model 3	7,171	-15.27 (-23.62, -6.92)	<0.0001
Model 4	5,966	-8.25 (-16.99, 0.50)	0.07
Phobia symptoms			
Model 1*	7,401	-9.82 (-14.75, -4.89)	<0.0001
Model 2	7,194	-8.47 (-13.48, -3.46)	0.001
Model 3	7,171	-7.51 (-12.44, -2.58)	0.003
Model 4	5,966	-4.15 (-9.25, 0.94)	0.11

* Model 1 was adjusted for gender & season. Model 2 was adjusted for gender, season & socioeconomic position. Model 3 was adjusted for gender, season & socioeconomic position and Body Mass Index. Model 4 was adjusted for gender, season, socioeconomic position, Body Mass Index, smoking and physical activity, PC/TV leisure time, suncover, blistering after sunburn, actively seeking suntan.

Appendix 3.12: Association between CMDs and 25(OH)D where 25(OH)D is the outcome: weighted-case results

	N	Coefficient (95% CI), % change in 25(OH)D	P_{trend}
Depressive symptoms			
Model 1*	7,154	-10.03 (-13.98, -6.07)	<0.0001
Model 2	7,004	-8.17 (-12.14, -4.20)	<0.0001
Model 3	6,983	-6.75 (-10.63, -2.86)	0.001
Model 4	5,817	-4.59 (-8.540, -0.63)	0.02
Anxiety symptoms			
Model 1*	7,154	-5.03 (-9.24, -0.81)	0.02
Model 2	7,004	-3.25 (-7.45, 0.95)	0.13
Model 3	6,983	-2.53 (-6.65, 1.60)	0.23
Model 4	5,817	0.34 (-3.76, 4.44)	0.87
Panic symptoms			
Model 1*	7,154	-19.77 (-29.05, -10.49)	<0.0001
Model 2	7,004	-17.78 (-27.42, -8.14)	<0.0001
Model 3	6,983	-15.20 (-24.89, -5.50)	0.002
Model 4	5,817	-9.02 (-18.74, 0.71)	0.07
Phobia symptoms			
Model 1*	7,154	-9.82 (-14.86, -4.79)	<0.0001
Model 2	7,004	-8.27 (-13.31, -3.22)	0.001
Model 3	6,983	-7.53 (-12.60, -2.46)	0.004
Model 4	5,817	-4.03 (-9.23, 1.17)	0.13

* Model 1 was adjusted for gender & season. Model 2 was adjusted for gender, season & socioeconomic position. Model 3 was adjusted for gender, season & socioeconomic position and Body Mass Index. Model 4 was adjusted for gender, season, socioeconomic position, Body Mass Index, smoking and physical activity, PC/TV leisure time, suncover, blistering after sunburn, actively seeking suntan.

Appendix 3.13: Examination of MHI-5 cut point ≤60 and ≤75: complete case results

	25- Hydroxyvitamin D, nmol/l						
	<25	25-49.9 OR (95% CI)	50-74.9 OR (95% CI)	75-99.9 OR (95% CI)	≥100 OR (95% CI)	P_{trend}	$P_{curvature}$
MHI-5 ≤60							
Model 1* (n=5966)	1.0	0.72 (0.57, 0.92)	0.55 (0.43, 0.70)	0.55 (0.41, 0.73)	0.49 (0.34, 0.69)	<0.001	0.001
Model 2 (n=5810)	1.0	0.76 (0.60, 0.98)	0.58 (0.45, 0.75)	0.58 (0.44, 0.78)	0.53 (0.37, 0.76)	<0.001	0.002
Model 3 (n=5810)	1.0	0.80 (0.62, 1.04)	0.62 (0.48, 0.81)	0.62 (0.46, 0.84)	0.60 (0.42, 0.88)	<0.001	0.002
Model 4 (n= 5793)	1.0	0.81 (0.63, 1.04)	0.64 (0.49, 0.83)	0.65 (0.48, 0.88)	0.63 (0.44, 0.92)	0.001	0.003
Model 5 (n=4964)	1.0	0.90 (0.68, 1.20)	0.71 (0.53, 0.97)	0.76 (0.54, 1.07)	0.80 (0.52, 1.22)	0.07	0.03
MHI-5 ≤75							
Model 1* (n=5966	1.0	0.78 (0.63, 0.97)	0.66 (0.53, 0.82)	0.63 (0.49, 0.80)	0.63 (0.47, 0.85)	<0.001	0.001
Model 2 (n=5810)	1.0	0.85 (0.66, 1.02)	0.70 (0.56, 0.88)	0.67 (0.52, 0.86)	0.68 (0.50, 0.82)	0.001	0.003
Model 3 n=5810)	1.0	0.86 (0.68, 1.07)	0.74 (0.59, 0.93)	0.71 (0.55, 0.91)	0.76 (0.56, 1.03)	0.01	0.004
Model 4 (n= 5793)	1.0	0.86 (0.69, 1.08)	0.78 (0.60, 0.95)	0.73 (0.57, 0.94)	0.79 (0.58, 1.07)	0.02	0.004
Model 5 (n=4964)	1.0	0.97(0.76, 1.25)	0.84(0.65, 1.10)	0.87 (0.65, 1.16)	0.91 (0.64, 1.30)	0.25	0.02

*Model 1 was adjusted for gender & season. Model 2 was adjusted for gender, season & socioeconomic position. Model 3 was adjusted for gender, season & socioeconomic position and presence of CMDS at 45 years. Model 4 was adjusted for gender, season, socioeconomic position presence of CMDS at 45 years and Body Mass Index, Model 5 was adjusted for gender, season, socioeconomic position presence of CMD at 45 years, BMI, smoking and physical activity, PC/TV leisure time, suncover, blistering after sunburn, actively seeking suntan.

Appendix 3.14: Examination of MHI-5 cut point ≤60 and ≤75: weighted-case results

	25- Hydroxyvitamin D, nmol/l					P_{trend}	$P_{curvature}$
	<25	25-49.9 OR (95% CI)	50-74.9 OR (95% CI)	75-99.9 OR (95% CI)	≥100 OR (95% CI)		
MHI-5 ≤60							
Model 1* (n=5772)	1.0	0.74(0.58, 0.95)	0.56 (0.43, 0.72)	0.53 (0.40, 0.71)	0.48 (0.33, 0.69)	<0.001	0.001
Model 2 (n=5657)	1.0	0.79 (0.62, 1.02)	0.60 (0.46, 0.78)	0.58 (0.43, 0.78)	0.53 (0.36, 0.77)	<0.001	0.002
Model 3 (n=5657)	1.0	0.83 (0.64, 1.08)	0.64 (0.49, 0.83)	0.61(0.45, 0.83)	0.60 (0.41, 0.88)	<0.001	0.002
Model 4 (n=5642)	1.0	0.83 (0.64, 1.08)	0.65 (0.50, 0.85)	0.64 (0.47, 0.87)	0.63 (0.43, 0.92)	0.001	0.003
Model 5 (n=4841)	1.0	0.90 (0.67, 1.21)	0.72 (0.53, 0.98)	0.73 (0.51, 1.03)	0.79 (0.51, 1.22)	0.05	0.02
MHI-5 ≤75							
Model 1* (n=5772)	1.0	0.80 (0.64, 0.99)	0.67 (0.54, 0.84)	0.62 (0.48, 0.80)	0.65 (0.48, 0.88)	<0.001	0.001
Model 2 (n= 5657)	1.0	0.84 (0.67, 1.05)	0.71 (0.57, 0.90)	0.67 (0.52, 0.86)	0.70 (0.51, 0.95)	0.001	0.003
Model 3 (n=5657)	1.0	0.87 (0.69, 1.09)	0.75 (0.59, 0.94)	0.70 (0.54, 0.91)	0.77(0.57, 1.06)	0.01	0.002
Model 4 (n=5642)	1.0	0.88 (0.70, 1.10)	0.76 (0.60, 0.96)	0.72 (0.56, 0.94)	0.80 (0.59, 1.10)	0.02	0.003
Model 5 (n=4841)	1.0	0.97 (0.75, 1.26)	0.85 (0.65, 1.12)	0.86 (0.63, 1.16)	0.92 (0.64, 1.32)	0.24	0.02

*Model 1 was adjusted for gender & season. Model 2 was adjusted for gender, season & socioeconomic position. Model 3 was adjusted for gender, season & socioeconomic position and presence of CMDS at 45 years. Model 4 was adjusted for gender, season, socioeconomic position presence of CMDS at 45 years and Body Mass Index, Model 5 was adjusted for gender, season, socioeconomic position presence of CMD at 45 years, BMI, smoking and physical activity, PC/TV leisure time, suncover, blistering after sunburn, actively seeking suntan.

Appendix 4 Additional information for Chapter 6

Appendix 4.1: PubMed search terms

(("Vitamin D"[MeSH Terms] OR cholecalciferol OR ergocalciferol OR "vitamin D" OR "vitamin D3" OR "vitamin D2" OR "Vitamin D"[Substance] OR "vitamin D deficiency"[mesh] OR Calcifediol[mesh] OR "25-hydroxyvitamin D"[text word] OR "25(OH)D"[text word])) AND ((((((((((((((Cognition) OR cognitive*) OR intelligence) OR learning) OR mental process) OR memory) OR recall) OR information processing) OR global impairment) OR attention) OR neuropsychological test*) OR problem solving) OR perception) OR spatial recognition) OR recognition).

Appendix 4.2: Additional results from vitamin D and cognition systematic review

Author (year), country	Total N	Setting (Study Name)	Groups	Adjustment
Cross-sectional				
McGrath (2007)(355), USA	11,232	Community (NHANES III)	1,676 adolescent 12-17years; 4,747 adult 20-60 years; 4809 elderly 60-90 years	Age, gender, activity, ethnicity
El-Ghoneimy (2009)(356), Egypt	30	Unknown	Patients with MS.	none
Hansen (2011)(357), Norway	25	Prison	NA	none
Tolppanen (2011)(358), USA	4932	Community (NHANES III)	Younger adults (20-59 years); Older adults (60-90 years)	Age, gender, race, health status, poverty-income ratio of household, education, outdoor physical activity, smoking alcohol
Lee (2009)(359), Italy, Belgium. Poland, Sweden, UK, Spain, Hungary, Estonia	3369	Community (EMAS)	NA	Age, education, BMI, physical activity, functional performance, mood/depression, season, smoking alcohol, centre
Seamans (2010)(360), Ireland, France, Rome, Italy	387	Community (ZENITH)	NA	Gender, age, BMI, zinc status, season

Author (year), country	Total N	Setting (Study Name)	Groups	Adjustment
Benge (2009)(361), USA	111	Hospital	39 PHPT with CSI; 72 PHPT without CSI	none
Oighara (1990)(362), Japan	60	Hospital	18 non-demented; 22 SDAT; 20 VTD	none
Houston (2007)(363), Italy	976	Community (InCHIANTI)	435 men; 541 women	Age
Llewellyn (2009)(364), UK	1766	Community and institutionalised (HSE)	212 cognitively impaired; 1554 cognitively normal	Age, education, ethnicity, season, smoking, alcohol, psychiatric morbidity, hypoalbuminemia, self-report medical history, impaired mobility
Llewellyn (2011)(365), USA	3325	Community (NHANES)	NA	Age, gender, ethnicity, education, season, smoking, BMI, alcohol, vitamin E, family income, impaired mobility, limited physical activity,
Aung (2006)(366), USA	44	Community (CREST)	Self-neglecting elders	none

Author (year), country	Total N	Setting (Study Name)	Groups	Adjustment
Buell (2009)(367), USA	1080	Community (NAME)	NA	Age, gender, race, education, BMI, kidney function, centre, season, physical activity, alcohol use, homocysteine, ApoE allele status, plasma B vitamins, multi-vitamin use
Buell (2010)(368), USA	318	Community (NAME)	Recipient of home care	Age, race, gender, BMI, education
Annweiler (2012)(369), France	125	Community (GAIT)	43 MCI; 52 CHI	Age, gender, BMI, number of comorbidities, education, MMSE score, Frontal Assessment Battery score, Geriatric Depression Scale, creatine clearance, season
Wilkins (2006)(324), USA	80	Community	40 mild dementia 40 non-demented	Age, race, gender
Wilkins (2009)(370), USA	60	Community	30 African Americans; 30 European Americans	Weight, gender, race, education, age
Annweiler (2010)(371), France	752	Community (EPIDOS)	NA	Age, BMI, number of chronic diseases, hypertension, depression, use of psychoactive drugs, education, physical activity, PTH, calcium

Author (year), country	Total N	Setting (Study Name)	Groups	Adjustment
Oudshoorn (2008)(372), Netherlands	225	Clinic	Outpatients with probable AD	Age, gender, mobility, action radius, education, vitamins b1, b6, b12
Perez-Llamas(2008)(373), Spain	86	Institutionalised	Nursing home	none
Przybelski (2007)(374), USA	80	Clinic	With cognitive symptoms from outpatient clinic	none
Skalska (2012)(375) Poland	140	Community, Hospital and Institution	62 Geriatric outpatient; 31 geriatric ward; 47 Institutionalised	
Annweiler (2010)(376), France	5596	Community (EPIDOS)	NA	BMI, sun exposure at midday, season, disability, number of chronic diseases, hypertension, depression, use of psychoactive drugs, education
Sakuma (2006)(377), Japan	50	Hospital	Acute hip fracture cases	

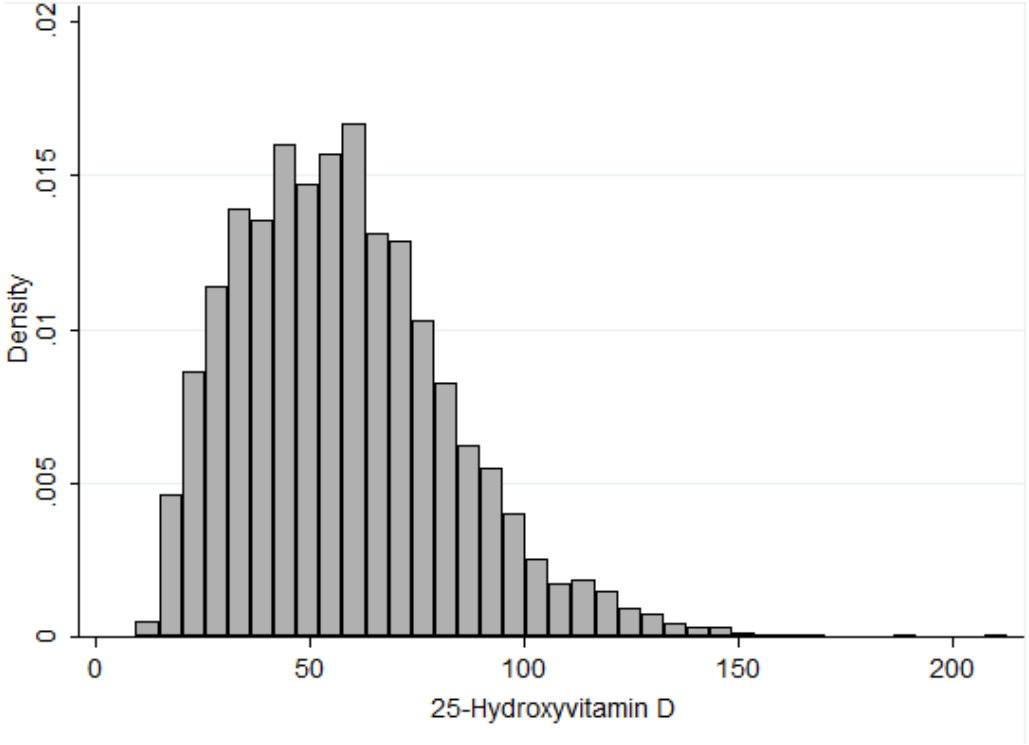
Author (year), country	Total N	Setting (Study Name)	Groups	Adjustment
Annweiler (2011)(378), France	228	Hospital	Geriatric inpatients	Age, gender, supine pulse pressure, number of acute and chronic diseases, psychoactive drugs, morphine, corticoids, PTH, albumin, creatine
Ravaglia (1998)(379), Italy	27	Community	15 dementia; 12 non-demented	Age, education
Case-control				
Evatt (2008)(380), USA	196	CRIN registry database	97 AD; 99 controls, matched on age, gender, race, APOE, residence	none
Martyn (1989)(381), UK	61	Hospital	27 AD; 34 controls	none
Walker (2009)(382), USA	128	Hospital Community	39 patients with PHPT underwent surgery 89 controls	Age. IQ, education, anxiety and depression
Jorde (2006)(333), Norway	84 (148 total cohort)	Community (Tromsø)	21 SHPT: 63 controls	none
Luckhaus (2009)(383), Germany	47	Outpatient clinic	20 AD; 19 MCI; 8 controls, matched on age	Gender
Ferrier (1990)(384), UK	50	Hospital & community	26 ATD cases 24 controls matched on age, gender	none
Sato (2005)(385), Japan	200	Clinic Community	58 severe AD; 48 mild AD; 100 matched controls	no

Author (year), country	Total N	Setting (Study Name)	Groups	Adjustment
Sato (1998)(386), Japan	186	Institutionalized Community	46 AD; 140 controls, matched on age	no
Cohort				
Chan (2011)(335), China	939	Community	939 baseline 629 follow-up, 4 years	Number of diseases, smoking, alcohol, BMI, physical activity, mobility limitations, dietary intake, season of blood measurement, serum PTH
Slinin (2010)(387), USA	1606	Community (MrOS)	Mean follow-up 4.6 years	Alcohol, Age, BMI, education, health,(self-reported), IADL, physical activity, race, season, site, smoking
Slinin (2012)(388), USA	6257	Community (Study of Osteoporotic Fractures)	Mean follow-up, 4 years	Clinic site, season, age at baseline, education, self-reported health, daily living impairments, smoking, BMI, hypertension, diabetes, depression, baseline cognitive function
Llewellyn (2010)(389), Italy	858	Community (InCHIANTI)	Mean follow-up 2 years	Alcohol, age, BMI, cognitive score(baseline), depressive symptoms, education, energy intake(total), impaired mobility, season, gender, smoking, vitamin E
Breitling (2012)(390), Germany	1639	Community (ESTHER)	987 women 652 men Follow-up 5 years	Age, education, BMI, season, smoking, alcohol, chronic disease (Cerebrovascular disease, depression, cancer

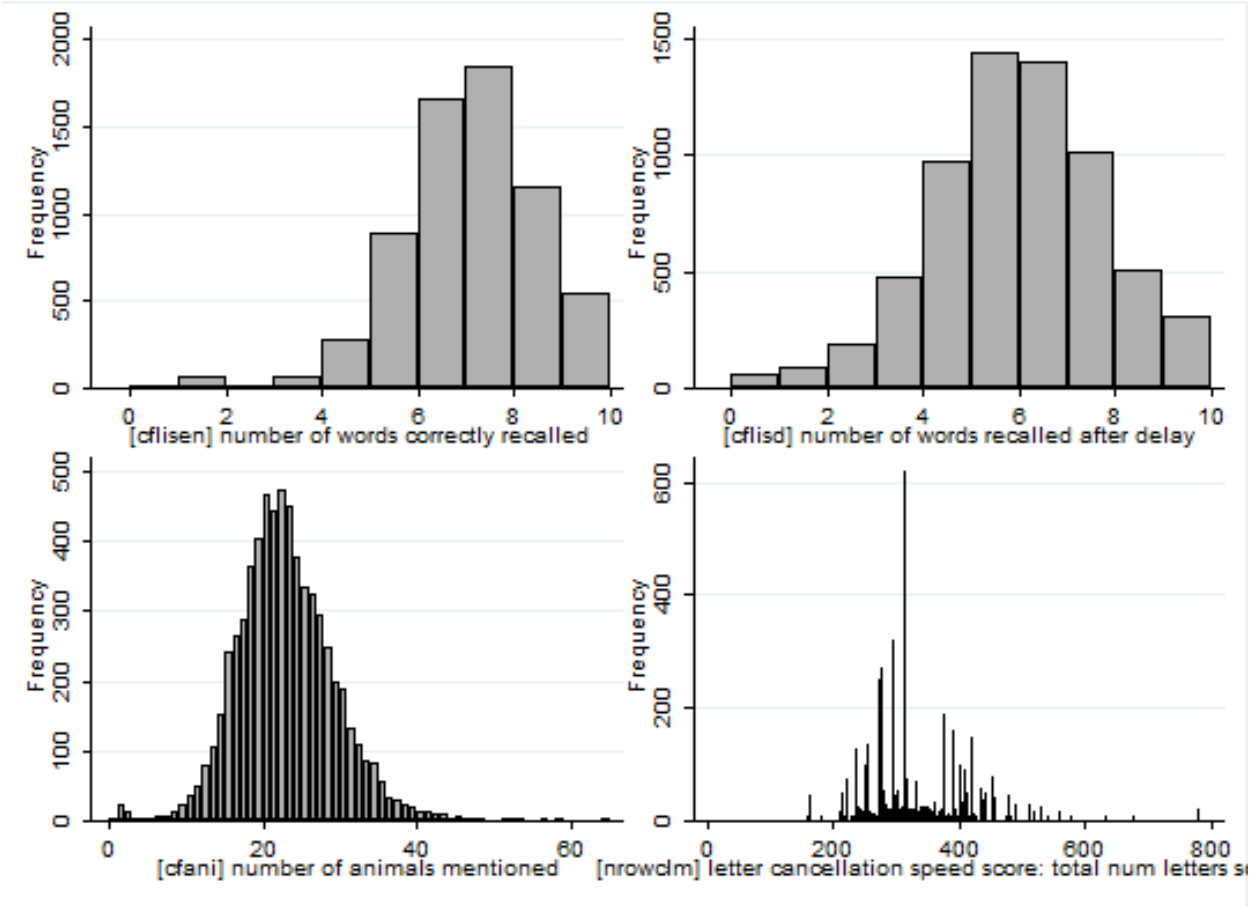
Author (year), country	Total N	Setting (Study Name)	Groups	Adjustment
Annweiler (2011)(391), France	40	Hospital Community	39 patients with PHPT underwent surgery 89 controls	Subtle cognitive impairments at baseline, presence of cardiovascular risk factors at baseline, and diagnosis of Parkinson's disease at baseline
Annweiler (2012)(392), France	498	Community (EPIDOS)	Follow-up 7 years	Age, AMI, baseline cognition, education, physical activity, sun exposure at mid-day, chronic diseases, hypertension, depression, psychoactive drugs
RCT				
Rossom (2012)(393), USA	4143	Women's Health Initiative mean 7.8 years	Treatment n=2034;1,000mg calcium carbonate & 400 IU vitamin D3 Control N=2109; Placebo	NA
Manders (2009)(394), Netherlands	176	Institution, 24 weeks	Treatment n=119; nutrient dense drink with 520IU vitamin D daily Control n=57; placebo drink no vitamins or minerals daily	NA
Chandra (2001)(395), Canada	96	Community, 12 months	Treatment n=48; 160IU vitamin D2 daily Control n=48; placebo tablets containing calcium and magnesium daily	NA
Before-after				
Annweiler (2012)(396), France	44	outpatients, 16 months	Treatment n=20; 800IU/d or 100,000 IU/month Control	Age, gender, cognitive score and baseline and between-visit time

Author (year), country	Total N	Setting (Study Name)	Groups	Adjustment
			n=24; unknown	
Przybelski (2008)(397), USA	63	Institution, 4 weeks	Treatment n=25; 50000IU oral D2 received 3 times per week Control n=38; placebo or increase of vitamin D supplementation over what receiving at baseline	

Appendix 4.3: Distribution of 25-Hydroxyvitamin D in the sample



Appendix 4.4: Distributions of cognitive outcomes used in this chapter, before standardisation



Appendix 4.5: Performance on cognitive tests according to participant characteristics

		Verbal memory				Verbal fluency		Speed of processing	
		Immediate word recall (Range 0-10)		Delayed word recall (Range 0-10)		Animal naming (Range 0-65)		Letter cancellation (Range 84-780)	
	Total*	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Gender									
Male	3,218	6.4	1.5	5.2	1.8	22.5	.5	321.3	55.1
Female	3,278	6.7	1.5	5.7	1.8	22.4	6.2	346.5	89.2
Combined		6.6	1.5	5.5	1.8	22.5	6.3	334.0	88.1
p		<0.001		<0.001		0.44		<0.001	
Region									
South	2,503	6.7	1.5	5.6	1.8	23.1	6.3	333.8	84.9
Middle	1,674	6.6	1.5	5.5	1.8	22.1	6.6	336.8	94.7
North	1,692	6.5	1.5	5.3	1.8	22.4	6.2	333.3	87.1
Scotland	621	6.4	1.6	5.5	1.9	21.1	6.0	329.3	85.2
Missing	6	6.8	1.2	5.5	1.7	26.8	4.9	339.8	78.6
p §		<0.001		<0.001		<0.001		0.3	
Socioeconomic position in adulthood†									
I or II	2,709	6.9	1.4	5.9	1.8	23.9	6.5	342.4	88.9
IIINM	1,373	6.7	1.4	5.6	1.8	22.0	6.1	340.0	89.9
IIIM	1,216	6.2	1.4	4.9	1.7	21.6	6.1	317.8	84.3
IV and V	976	6.2	1.5	5.0	1.8	20.7	.8	324.9	84.6
Other/unknown	222	6.2	1.6	4.9	2.0	21	6.	323.0	86.8
p §		<0.001		<0.001		0.001		<0.001	
BMI(kg/m2)‡									
<30	4,920	6.6	1.5	5.5	1.8	22.6	6.4	335.7	89.1
≥30	1,546	6.5	1.4	5.4	1.8	21.9	6.2	328.9	84.7
Missing	30	6.6	1.6	4.9	1.9	21.9	6.7	318.4	80.6
p§		0.002		0.01		<0.001		0.0	
Menopause‡									
Pre-menopausal	2,099	6.6	1.4	5.7	1.8	22.5	6.2	345.5	88.5
Peri-menopausal	601	6.8	1.4	5.9	1.7	22.7	6.2	344.9	84.4
Post-menopausal	123	6.6	1.5	5.5	1.8	21.7	6.9	337.7	89.3

		Verbal memory				Verbal fluency		Speed of processing	
Other reasons	388	6.6	1.6	5.6	1.9	21.8	5.8	354.4	97.1
Missing	66	6.2	1.9	5.4	2.0	20.7	5.4	359.4	101.5
p		<0.001		<0.001		0.74		<0.001	
Physical activity (times/week) †									
≤1	3,323	6.5	1.5	5.4	1.8	22.4	6.4	330.5	86.4
2-3	1,373	6.7	1.4	5.6	1.8	23.0	6.4	336.3	88.9
4-7	1,656	6.6	1.5	5.5	1.8	22.7	6.1	338.9	89.6
Missing	144	6.3	1.6	5.2	1.8	22.5	5.9	336.7	98.8
p §		<0.001		<0.001		<0.001		<0.00	
Smoking†									
Never/Former	4,911	6.7	1.5	5.6	1.8	22.8	6.3	335.5	87.7
Current	1,433	6.3	1.5	5.2	1.8	21.4	6.2	328.9	88.3
Missing	152	6.3	1.7	5.2	2.0	22.5	6.0	334.3	95.7
p §		<0.001		<0.001		<0.001		0.01	
Alcohol (units/week)l‡									
Non-drinker	5,154	6.2	1.5	4.9	1.8	21.5	6.4	336.4	93.0
Light (<7)	1,319	6.6	1.5	5.5	1.8	22.1	6.3	332.9	86.2
Moderate (7-14)	1,660	6.7	1.5	5.7	1.8	22.9	6.3	338.5	90.7
Heavy (14-21)	735	6.7	1.4	5.5	1.8	23.3	6.3	334.9	88.0
Very heavy (>21)	584	6.4	1.6	5.4	1.8	23.0	6.4	324.4	85.6
Missing	23	6.3	1.8	4.9	2.2	21.7	6.0	336.5	121.
p §		<0.001		<0.001		<0.001		0.01	
Depressive symptoms‡									
<2 symptoms	5,990	6.6	1.5	5.5	1.8	22.5	6.3	334.2	87.7
≥2 symptoms	481	6.4	(1.5	5.2	1.9	21.5	6.1	331.1	90.5
Missing	25	6.6	1.3	5.6	2.0	23.6	4.6	342.7	130.1
p§		<0.001		<0.001		<0.001		0.23	

* N varies according to test; 6,469 for immediate word recall and animal naming, 6,454 for delayed word recall, 6378 for letter cancellation.
† Data obtained when participants were aged 42 years
‡ Data obtained when participants were aged 45 years
§p value from linear regression with standardised cognitive outcomes, adjusted for gender

Appendix 4.6: Association between early life factors and standardised cognitive tests at 50 years

	Immediate word recall		Delayed word recall		Animal naming		Letter cancellation	
	Coef	(95% CI)	Coef	(95% CI)	Coef	(95% CI)	Coef	(95% CI)
Educational attainment*	0.21	(0.19, 0.23)	0.20	(0.18, 0.22)	0.22	(0.20, 0.24)	0.07	(0.05, 0.09)
p ‡	<0.001				<0.001		<0.001	
Childhood cognition†	0.45	(0.43, 0.48)	0.43	(0.40, 0.46)	0.44	(0.41, 0.47)	0.12	(0.09, 0.15)
p ‡	<0.001		<0.001		<0.001		<0.001	

*N varies according to test; 6,496 for immediate word recall and animal naming, 6,454 for delayed word recall, 6,378 for letter cancellation
† N varies according to test; 6,428 for immediate word recall and animal naming, 6,386 for delayed word recall, 6,312 for letter cancellation
‡ p value from linear regression adjusted for gender

Appendix 4.7: Association between 25(OH)D at 45 years and standardised cognitive function 50 years: complete-case results

	25- Hydroxyvitamin D, nmol/l										
	<25	25-49.9		50-74.9		75-99.9		≥100		p_{trend}	$p_{curvature}$
		Coef	95% CI	Coef	95% CI	Coef	95% CI	Coef	95% CI		
Immediate word recall *											
Model 1† (n=6,496)	1.0	0.12	(0.02 to 0.21)	0.16	(0.06 to 0.26)	0.15	(0.04 to 0.26)	-0.02	(-0.16 to 0.11)	0.76	<0.001
Model 2‡ (n=6,303)	1.0	0.09	(0.00 to 0.19)	0.12	(0.02 to 0.21)	0.10	(-0.00 to 0.21)	-0.05	(-0.12 to 0.09)	0.68	0.001
Model 3§ (n=6,039)	1.0	0.08	(-0.01 to 0.18)	0.08	(-0.01 to 0.18)	0.06	(-0.05 to 0.17)	-0.07	(-0.21 to 0.06)	0.19	0.004
Delayed word recall *											
Model 1† (n=6,454)	1.0	0.06	(-0.04 to 0.15)	0.09	(-0.01 to 0.19)	0.06	(-0.05 to 0.17)	-0.08	(-0.21 to 0.05)	0.44	0.01
Model 2‡ (n=6,264)	1.0	0.04	(-0.05 to 0.13)	0.06	(-0.03 to 0.16)	0.03	(-0.07 to 0.14)	-0.07	(-0.20 to 0.06)	0.35	0.06
Model 3§ (n=6,000)	1.0	0.05	(-0.05 to 0.14)	0.05	(-0.05 to 0.15)	0.02	(-0.09 to 0.13)	-0.08	(-0.21 to 0.05)	0.16	0.08
Animal naming *											
Model 1† (n=6,496)	1.0	0.14	(0.04 to 0.24)	0.17	(0.06 to 0.27)	0.14	(0.03 to 0.25)	0.16	(0.02 to 0.30)	0.10	0.06
Model 2‡ (n=6,303)	1.0	0.11	(0.02 to 0.21)	0.11	(0.02 to 0.21)	0.08	(-0.03 to 0.19)	0.12	(-0.01 to 0.25)	0.46	0.31
Model 3§ (n=6,039)	1.0	0.10	(0.00 to 0.20)	0.10	(-0.01 to 0.20)	0.05	(-0.06 to 0.17)	0.09	(-0.04 to 0.23)	0.90	0.37
Letter cancellation (men) *											
Model 1† (n=3,156)	1.0	-0.01	(-0.15 to 0.15)	0.07	(-0.09 to 0.22)	0.10	(-0.06 to 0.27)	0.07	(-0.13 to 0.26)	0.07	0.21
Model 2‡ (n=3,060)	1.0	-0.02	(-0.17 to 0.13)	0.05	(-0.11 to 0.20)	0.08	(-0.09 to 0.25)	0.07	(-0.12 to 0.27)	0.06	0.26
Model 3 (n=2,955)	1.0	-0.03	(-0.19 to 0.12)	0.03	(-0.13 to 0.19)	0.06	(-0.12 to 0.24)	0.08	(-0.13 to 0.28)	0.09	0.48
Letter cancellation (women) *											
Model 1† (n=3,222)	1.0	-0.05	(-0.18 to 0.08)	0.04	(-0.10 to 0.17)	-0.05	(-0.20 to 0.10)	0.01	(-0.19 to 0.20)	0.73	0.23
Model 2‡ (n=3,127)	1.0	-0.06	(-0.19 to 0.08)	0.04	(-0.10 to 0.18)	-0.06	(-0.21 to 0.10)	-0.02	(-0.22 to 0.17)	0.86	0.17
Model 3§ (n=2,974)	1.0	-0.06	(-0.20 to 0.08)	-0.00	(-0.15 to 0.15)	-0.13	(-0.30 to 0.03)	-0.07	(-0.28 to 0.13)	0.33	0.36

Coef, Beta-coefficient from linear regression model

*N for immediate word recall varied from 6,496 to 6,039 for final model. N for delayed word recall varied from 6,454 to 6,000 for final model). N for animal varied from 6,496 to 6,039. N for letter cancellation (men) varied from 3,156 to 2,955 for final model. N for letter cancellation (women) varied from 3,222 to 2,974.

†adjusted for gender, season, day and time of cognitive testing, presence of others in the room, other contextual factors affecting performance (i.e. blind or poor eyesight, deaf or hard of hearing, too tired, illness or physical impairment that affects ability to perform, impaired concentration, very nervous or anxious, mental impairment, interruption or distraction, noisy environment, problems with the laptop or difficulty understanding English), word list & method of delivery (latter two for immediate & delayed word recall only);

‡additionally adjusted for region, SEP at 42 years, SEP at birth (7 years if missing), childhood cognition and educational attainment by 42 years;

§additionally adjusted for obese BMI, menopausal status; smoking, alcohol, physical activity; depressive symptoms at 45 years.

Appendix 4.8: Association between 25(OH)D at 45 years and standardised cognitive function 50 years: weighed-case results

	25- Hydroxyvitamin D, nmol/l									
	<25	25-49.9		50-74.9		75-99.9		≥100		ptrend	pcurvature
		Coef	95% CI	Coef	95% CI	Coef	95% CI	Coef	95% CI		
Immediate word recall *											
Model 1† (n=6,286)	1.0	0.12	(0.01 to 0.23)	0.17	(0.05 to 0.28)	0.16	(0.04 to 0.28)	-0.02	(-0.17 to 0.12)	0.79	<0.001
Model 2 (n=6,303)‡	1.0	0.09	(-0.01 to 0.20)	0.11	(0.01 to 0.22)	0.10	(-0.01 to 0.22)	-0.04	(-0.19 to 0.10)	0.71	0.01
Model 3 (n=6,039)§	1.0	0.08	(-0.03 to 0.19)	0.08	(-0.03 to 0.19)	0.06	(-0.06 to 0.18)	-0.08	(-0.23 to 0.07)	0.20	0.01
Delayed word recall *											
Model 1† (n=6,244)	1.0	0.06	(-0.04 to 0.17)	0.09	(-0.02 to 0.19)	0.07	(-0.05 to 0.18)	-0.09	(-0.23 to 0.05)	0.38	0.01
Model 2 (n=6,157)‡	1.0	0.04	(-0.06 to 0.14)	0.05	(-0.05 to 0.16)	0.03	(-0.08 to 0.15)	-0.08	(-0.22 to 0.05)	0.30	0.05
Model 3 (n=5,899)§	1.0	0.05	(-0.06 to 0.15)	0.05	(-0.06 to 0.15)	0.02	(-0.10 to 0.14)	-0.09	(-0.23 to 0.05)	0.13	0.07
Animal naming *											
Model 1† (n=6,286)	1.0	0.14	(0.04 to 0.25)	0.17	(0.06 to 0.27)	0.14	(0.03 to 0.26)	0.14	(0.00 to 0.28)	0.14	0.08
Model 2 (n=6,196)‡	1.0	0.11	(0.01 to 0.21)	0.11	(0.01 to 0.21)	0.08	(-0.03 to 0.19)	0.11	(-0.02 to 0.25)	0.52	0.34
Model 3 (n=5,938)§	1.0	0.10	(-0.01 to 0.20)	0.09	(-0.01 to 0.20)	0.06	(-0.06 to 0.18)	0.09	(-0.01 to 0.15)	0.90	0.40
Letter cancellation (men) *											
Model 1† (n=3,053)	1.0	0.01	(-0.15 to 0.17)	0.08	(-0.08 to 0.24)	0.13	(-0.05 to 0.30)	0.09	(-0.12 to 0.30)	0.04	0.28
Model 2 (n=3,003)‡	1.0	0.01	(-0.16 to 0.16)	0.07	(-0.09 to 0.23)	0.11	(-0.06 to 0.29)	0.11	(-0.10 to 0.32)	0.04	0.35
Model 3 (n=2,901)§	1.0	-0.01	(-0.18 to 0.16)	0.05	(-0.12 to 0.22)	0.10	(-0.09 to 0.28)	0.11	(-0.11 to 0.33)	0.05	0.55
Letter cancellation (women) *											
Model 1† (n=3,120)	1.0	-0.07	(-0.21 to 0.08)	0.03	(-0.12 to 0.17)	-0.06	(-0.23 to 0.10)	-0.04	(-0.23 to 0.15)	0.94	0.12
Model 2 (n=3,080)‡	1.0	-0.06	(-0.21 to 0.08)	0.03	(-0.12 to 0.18)	-0.05	(-0.22 to 0.12)	-0.05	(-0.24 to 0.15)	0.93	0.10
Model 3 (n=2,929)§	1.0	-0.07	(-0.22 to 0.08)	-0.01	(-0.16 to 0.15)	-0.14	(-0.31 to 0.03)	-0.10	(-0.31 to 0.10)	0.26	0.26

Coef, Beta-coefficient from linear regression model

*N for immediate word recall varied from 6,286 to 5,938 for final model. N for delayed word recall varied from 6,244 to 5,899 for final model. N for animal naming varied from 6,286 to 5,938 for final model. N for letter cancellation (men) varied from 3,053 to 2,901 for final model. N for letter cancellation (women) cared from 3,120 to 2,929 for final model.

†adjusted for gender, season, day and time of cognitive testing, presence of others in the room, other contextual factors affecting performance (i.e. blind or poor eyesight, deaf or hard of hearing, too tired, illness or physical impairment that affects ability to perform, impaired concentration, very nervous or anxious, mental impairment, interruption or distraction, noisy environment, problems with the laptop or difficulty understanding English), word list & method of delivery (latter two for immediate & delayed word recall only);

‡additionally adjusted for region, SEP at 42 years, SEP at birth (7 years if missing), childhood cognition and educational attainment by 42 years;

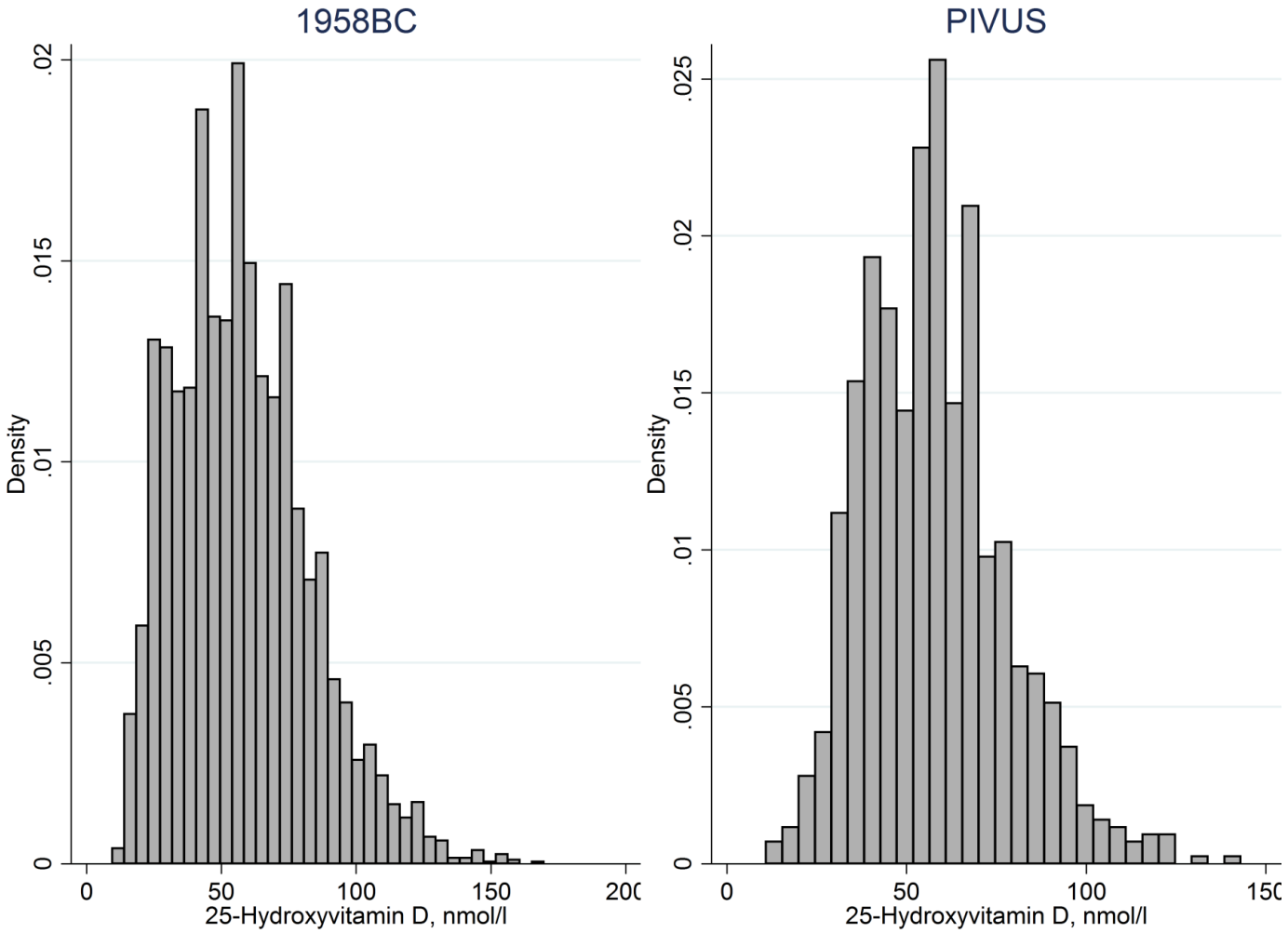
§additionally adjusted for obese BMI, menopausal status; smoking, alcohol, physical activity; depressive symptoms at 45 years.

Appendix 5: Additional information for Chapter 7

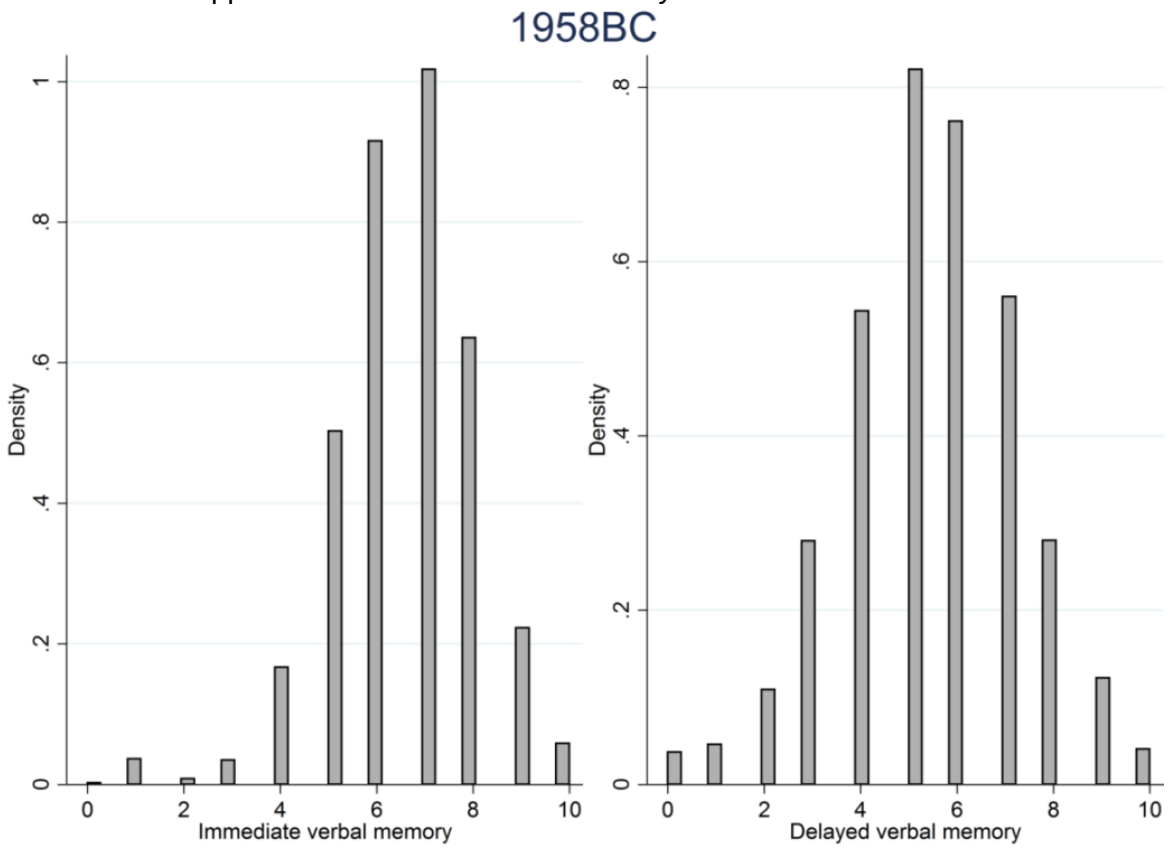
Appendix 5.1: SNP quality control

Study	SNP	Alleles Measured	Call Rate	MAF	HWE	Imputed or genotyped?
1958BC	rs7412	C/T	99.98	0.08	0.64	Imputed
	rs429358	T/C	99.86	0.16	0.19	Genotyped
PIVUS	rs7412	C/T	100.00	0.08	0.82	Genotyped
	rs429358	T/C	99.47	0.16	1.00	Genotyped

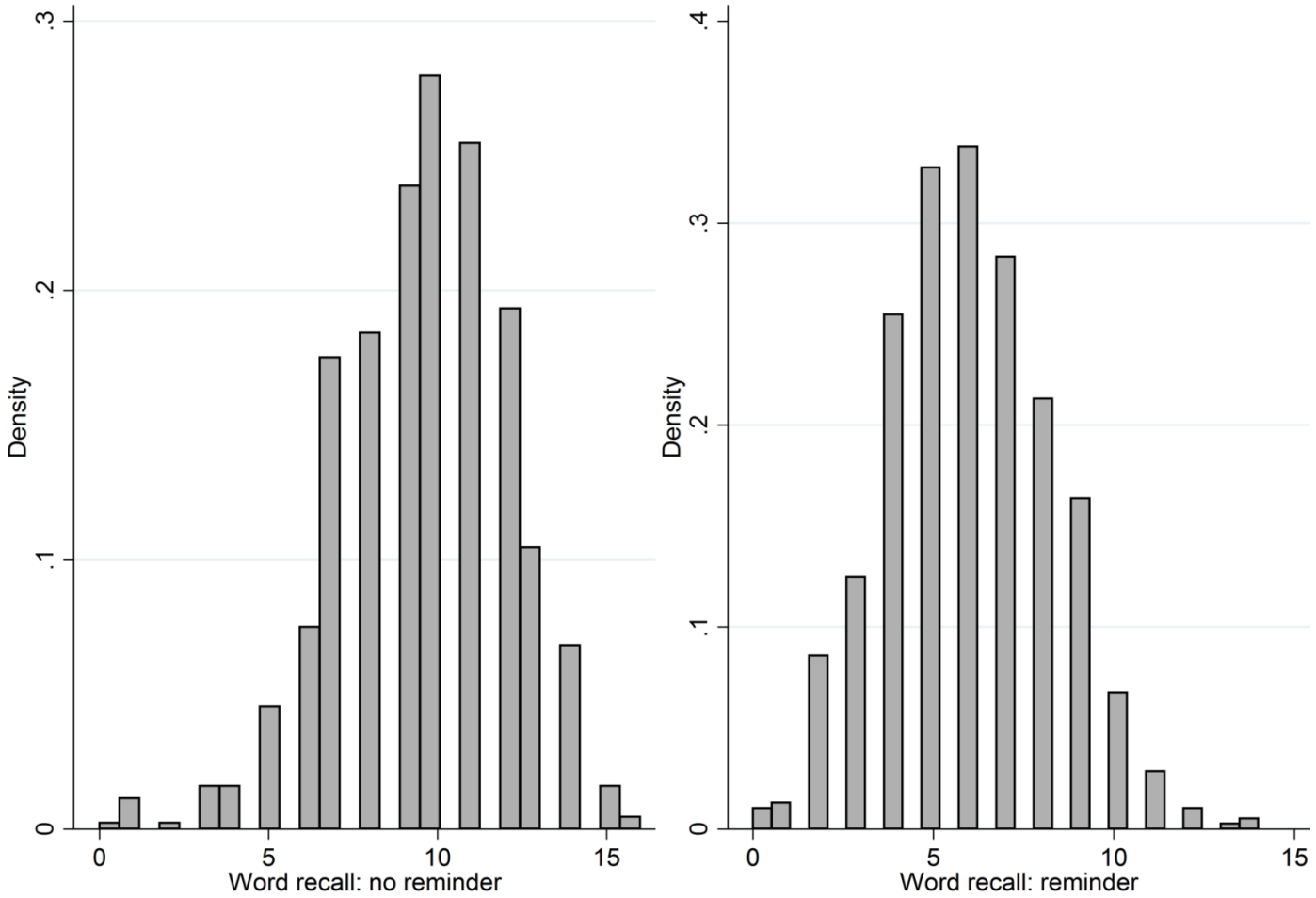
Appendix 5.2: Distribution of 25(OH)D in each cohort



Appendix 5.3: Distribution of memory function in each cohort



PIVUS


Density
0
.1
.2
.3
0
5
10
15
Word recall: no reminder
Density
0
.1
.2
.3
.4
0
5
10
15
Word recall: reminder

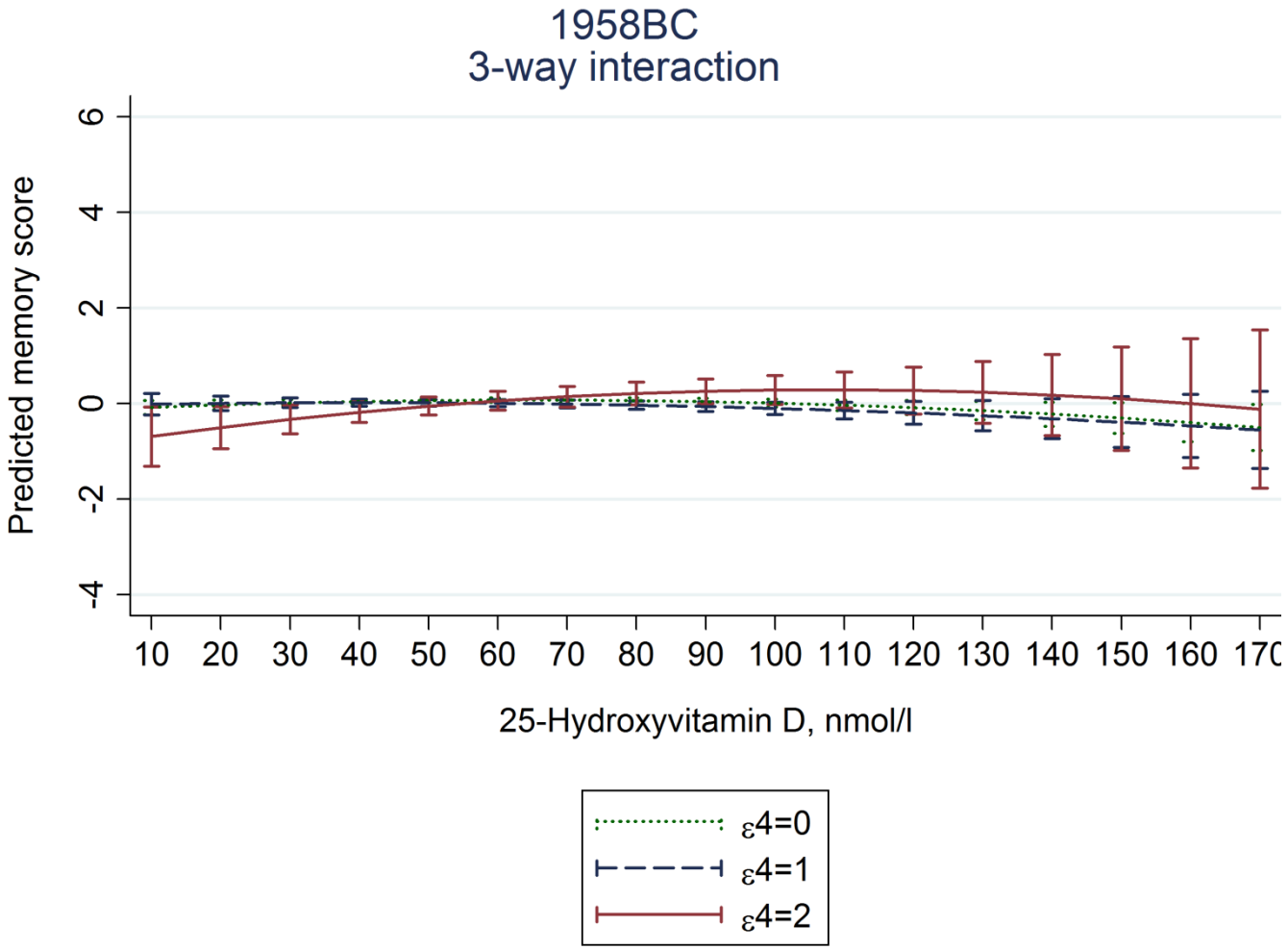
Appendix 5.4: Association between *APOE* ε4 and naturally log-transformed 25(OH)D

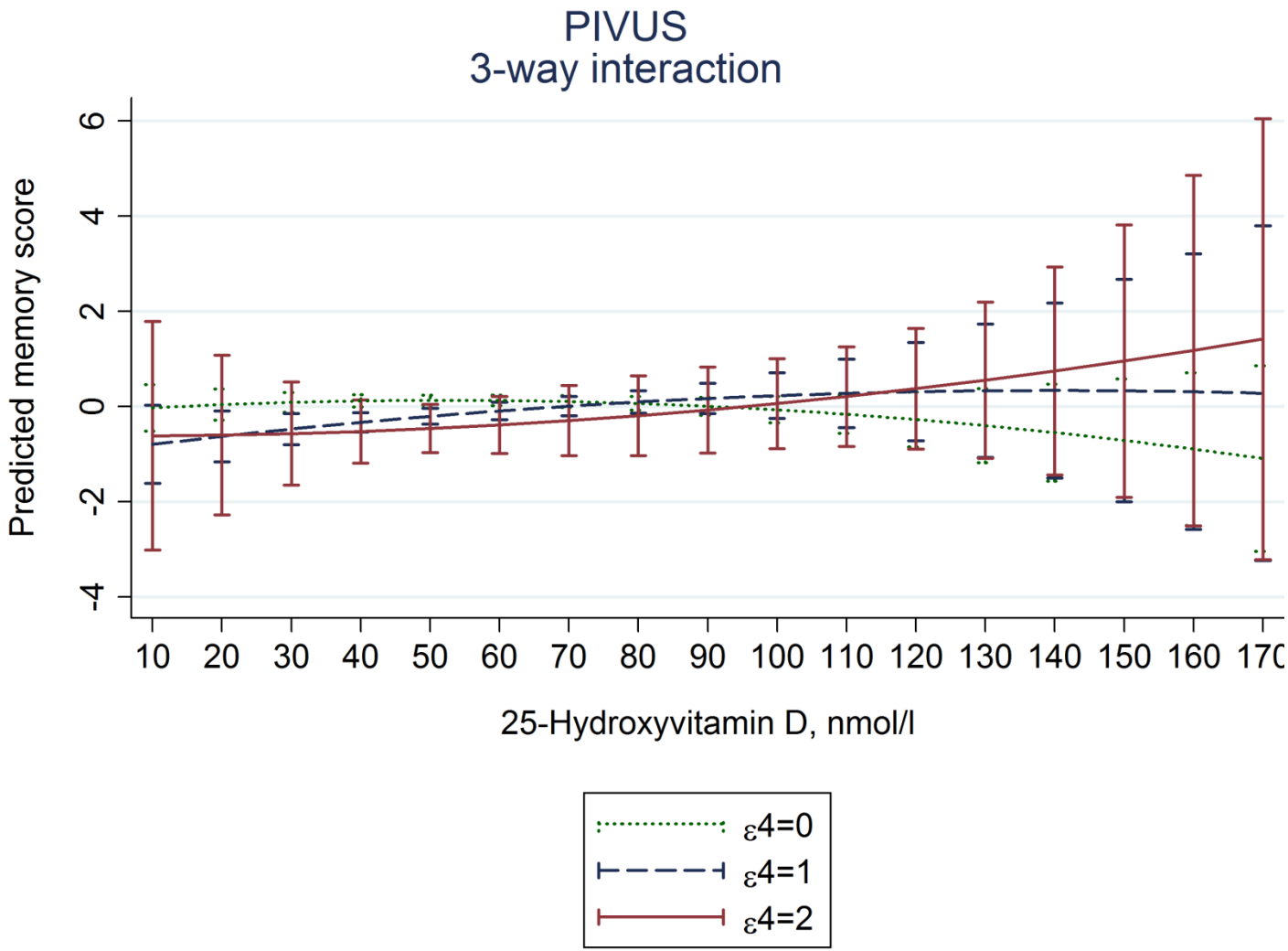
		25-Hydroxyvitamin D (nmol/l)			
		Model 1		Model 2	
	N(%)	Coefficient	(95% CI)	Coefficient	(95% CI)
1958BC					
zero *APOE* ε4 alleles	2,787 (68.2)	Reference	Reference	Reference	Reference
one *APOE* ε4 alleles	1,158 (28.4)	-0.01	(-0.04 to 0.02)	-0.01	(-0.04 to 0.02)
two *APOE* ε4 alleles	139 (3.4)	0.06	(-0.01 to 0.13)	0.06	(-0.01 to 0.13)
		p_{trend} = 0.56		p_{trend} =0.55	
PIVUS					
zero *APOE* ε4 alleles	549 (68.5)	Reference	Reference	Reference	Reference
one *APOE* ε4 alleles	229 (28.6)	-0.03	(-0.09 to 0.03)	-0.02	(-0.09 to 0.04)
two *APOE* ε4 alleles	24 (3.0)	-0.06	(-0.23 to 0.10)	-0.06	(-0.22 to 0.11)
		p_{trend} =0.27		p_{trend} =0.32	

Model 1 adjusted for age (in PIVUS), gender, month, region (for 1958BC):*n*=3,913 for 1958BC, *n*=629 for PIVUS;

Model 2 adjusted for age (in PIVUS), gender, month, region (for 1958BC), standardised memory score: *n*=3,913 for 1958BC, *n*=629 for PIVUS

Appendix 5.5: 3-way interaction between 25(OH)D, 25(OH)D^2 and number of APOE ε4 alleles




PIVUS
3-way interaction
Predicted memory score
6
4
2
0
-2
-4
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
25-Hydroxyvitamin D, nmol/l
ε4=0
ε4=1
ε4=2

Appendix 5.6: Association between 25(OH)D and memory, stratified by number of *APOE* ε4 alleles

			Standardised memory score					
			zero *APOE* ε4 alleles*		one *APOE* ε4 alleles†		two *APOE* ε4 alleles‡	
25(OH)D, nmol/l			Coefficient	(95% CI)	Coefficient	(95% CI)	Coefficient	(95% CI)
1958BC								
	<25	328 (7.1)	Reference	Reference	Reference	Reference	Reference	Reference
	25-49	1,570 (33.8)	0.09	(-0.08, 0.26)	-0.14	(-0.39, 0.10)	0.41	(-0.48, 1.30)
	50-74	1,732 (37.2)	0.13	(-0.04, 0.30)	-0.03	(-0.28, 0.23)	0.70	(-0.22, 1.62)
	≥75	1,014 (21.9)	0.05	(-0.14, 0.24)	-0.17	(-0.44, 0.11)	0.77	(-0.23, 1.77)
			p_{trend} =0.86		p_{trend} =0.65		p_{trend} =0.10	
			$P_{curvature}$ = 0.03		$P_{curvature}$ = 0.24		$P_{curvature}$ = 0.18	
PIVUS								
	<25	16 (2.2)	Reference	Reference	Reference	Reference	Reference	Reference
	25-49	253 (34.4)	0.02	(-0.39, 0.44)	0.60	(-0.76, 1.95)	-8.88	(-18.51, 0.75)
	50-74	328 (44.6)	-0.03	(-0.44, 0.38)	0.99	(-0.38, 2.35)	-5.07	(-12.37, 2.24)
	≥75	139 (18.9)	-0.04	(-0.47, 0.39)	1.13	(-0.26, 2.53)	omitted	omitted
	P_{trend}		p_{trend} =0.47		p_{trend} =0.02		p_{trend} =0.04	
			$P_{curvature}$ = 0.20		$P_{curvature}$ = 0.58		$P_{curvature}$ = 0.09	

Coefficient from linear regression model;
adjusted for age (PIVUS) gender, month of blood collection, region (in 1958BC), education and depressive symptoms (in 1958BC):
* *n*= 2,328 for 1958BC, *n*= 430 for PIVUS.
† *n*= 972 for 1958BC, *n*= 176 for PIVUS.
‡ *n*=126 for 1958BC, *n*=17 for PIVUS.

Appendix 5.7: Association between 25(OH)D and memory, stratified by recessive model of *APOE* ε4 alleles

		Standardised memory score			
		zero or one *APOE* ε4 alleles*		two *APOE* ε4 alleles†	
25(OH)D, nmol/l		Coefficient	(95% CI)	Coefficient	(95% CI)
1958BC					
<25	328 (7.1)	Reference	Reference	Reference	Reference
25-49	1,570 (33.8)	0.03	(-0.11, 0.18)	0.41	(-0.48, 1.30)
50-74	1,732 (37.2)	0.09	(-0.05, 0.23)	0.70	(-0.22, 1.62)
≥75	1,014 (21.9)	-0.01	(-0.16, 0.15)	0.77	(-0.23, 1.77)
		P_{trend} = 0.96		P_{trend} = 0.10	
		$P_{curvature}$ = 0.02		$P_{curvature}$ = 0.08	
PIVUS					
<25	16 (2.2)	Reference	Reference	Reference	Reference
25-49	253 (34.4)	0.17	(-0.31, 0.66)	-8.88	(-18.51, 0.75)
50-74	328 (44.6)	0.22	(-0.26, 0.71)	-5.07	(-12.37, 2.24)
≥75	139 (18.9)	0.29	(-0.21, 0.79)	omitted	omitted
		P_{trend} = 0.19		P_{trend} = 0.04	
		$P_{curvature}$ = 0.29		$P_{curvature}$ = 0.09	

Coefficient from linear regression model;
adjusted for age (PIVUS) gender, month of blood collection, region (in 1958BC), education and depressive symptoms (in 1958BC):
* *n*=3,300 for 1958BC, *n*=606 for PIVUS.
† *n*=126 for 1958BC, *n*=17 for PIVUS.

Appendix 5.8: Sensitivity analyses: Association between 25(OH)D and standardised memory function score

		Standardised memory function score			
		Model 1*		Model 2†	
25-hydroxyvitamin D, nmol/l	N(%)	Coefficient	(95% CI)	Coefficient	(95% CI)
1958BC					
<25	267 (6.82)	Reference	Reference	Reference	Reference
25-49	1,359 (34.72)	0.04	(-0.10, 0.17)	0.04	(-0.10, 0.17)
50-74	1,443 (36.87)	0.10	(-0.04, 0.24)	0.10	(-0.04, 0.24)
≥75	845 (21.59)	0.01	(-0.14, 0.17)	0.01	(-0.14, 0.17)
		P_{trend} = 0.85		P_{trend} = 0.83	
		$P_{curvature}$ = 0.02		$P_{curvature}$ = 0.02	
PIVUS					
<25	15 (2.38)	Reference	Reference	Reference	Reference
25-49	219 (34.82)	0.19	(-0.34, 0.73)	0.22	(-0.32, 0.75)
50-74	276 (43.88)	0.22	(-0.31, 0.75)	0.22	(-0.30, 0.75)
≥75	119 (18.92)	0.30	(-0.25, 0.85)	0.32	(-0.25, 0.86)
		P_{trend} = 0.26		P_{trend} = 0.29	
		$P_{curvature}$ = 0.37		$P_{curvature}$ = 0.32	

Beta-coefficient from linear regression model;
* adjusted for age (PIVUS) gender, month of blood collection, region (in 1958BC), education and depressive symptoms (in 1958BC): *n*= 3,426 for 1958BC, *n*=623for PIVUS.
† adjusted for age (PIVUS), gender, month, region (in 1958BC), education and depressive symptoms (in 1958BC) and number of *APOE* ε4 alleles: *n*=3,426 for 1958BC, *n*=623 for PIVUS

Appendix 5.9: Sensitivity analyses: Association between *APOE* ε4 and naturally log-transformed 25(OH)D

		25-Hydroxyvitamin D (nmol/l)			
		Model 1		Model 2	
	N(%)	Coefficient	(95% CI)	Coefficient	(95% CI)
1958BC					
zero *APOE* ε4 alleles	2,667 (68.14)	Reference	Reference	Reference	Reference
one *APOE* ε4 alleles	1,109 (28.33)	-0.01	(-0.04, 0.02)	-0.01	(-0.04, 0.02)
two *APOE* ε4 alleles	138 (3.53)	0.06	(-0.01, 0.13)	0.06	(-0.01, 0.13)
		p_{trend} = 0.56		p_{trend} =0.55	
PIVUS					
zero *APOE* ε4 alleles	433 (68.84)	Reference	Reference	Reference	Reference
one *APOE* ε4 alleles	178 (28.30)	-0.03	(-0.09, 0.03)	-0.02	(-0.09, 0.04)
two *APOE* ε4 alleles	18 (2.86)	-0.06	(-0.23, 0.10)	-0.06	(-0.22, 0.11)
		p_{trend} =0.27		p_{trend} = 0.32	

Model 1 adjusted for age (in PIVUS), gender, month, region (for 1958BC):*n*= 3,913 for 1958BC, *n*= 629 for PIVUS;
Model 2 adjusted for age (in PIVUS), gender, month, region (for 1958BC), standardised memory score: *n*= 3,913 for 1958BC, *n*= 629 for PIVUS

Appendix 5.10: Sensitivity analyses: Association between *APOE* ε4 and memory score

		Standardised memory scores			
		Model 1*		Model 2 †	
	N(%)	Coefficient	(95% CI)	Coefficient	(95% CI)
1958BC					
zero *APOE* ε4 alleles‡	2,667 (68.14)	Reference	Reference	Reference	Reference
one *APOE* ε4 alleles	1,109 (28.33)	-0.05	(-0.12, 0.02)	-0.05	(-0.12, 0.02)
two *APOE* ε4 alleles	138 (3.53)	-0.05	(-0.21, 0.12)	-0.04	(-0.21, 0.13)
		p_{trend} = 0.18		p_{trend} =0.18	
PIVUS					
zero *APOE* ε4 alleles‡	433 (68.84)	Reference	Reference	Reference	Reference
one *APOE* ε4 alleles	178 (28.30)	-0.25	(-0.42, -0.08)	-0.25	(-0.42, -0.07)
two *APOE* ε4 alleles	18 (2.86)	-0.45	(-0.92, 0.03)	-0.45	(-0.92, 0.03)
		p_{trend} =0.001		p_{trend} =0.001	

*adjusted for age (in PIVUS), gender, region (for 1958BC), education and depressive symptoms (in 1958BC):*n*= 3,426 for 1958BC, *n*= 623 for PIVUS;
†adjusted for age (in PIVUS), gender, region (for 1958BC), education, depressive symptoms (in 1958BC) and 25(OH)D concentrations: *n*= 3,426 for 1958BC, *n*= 623 for PIVUS
‡two ε3 alleles

1958BC

Three-way interaction between 25(OH)D, 25(OH)D^2 and number of *APOE* ε4 alleles was not significant (overall $p_{interaction}$=0.75). However the curvature term (25(OH)D^2) remained significant in this GxE model (*p*=0.02). Therefore, a two-way GxE model was examined, adjusting for the curvature term. The two-way GxE, adjusting for the curvature term was significant (overall $p_{interaction}$=0.02).

PIVUS

There was no evidence of three-way interaction (overall $p_{interaction}$=0.87). Although the curvature term was not significant in this model (*p*=0.31), a two-way GxE model, adjusting for the curvature term was applied to be comparable with GxE models of the 1958BC. There was evidence of GxE interaction using the adjusted two-way interaction model (overall $p_{interaction}$=0.02).

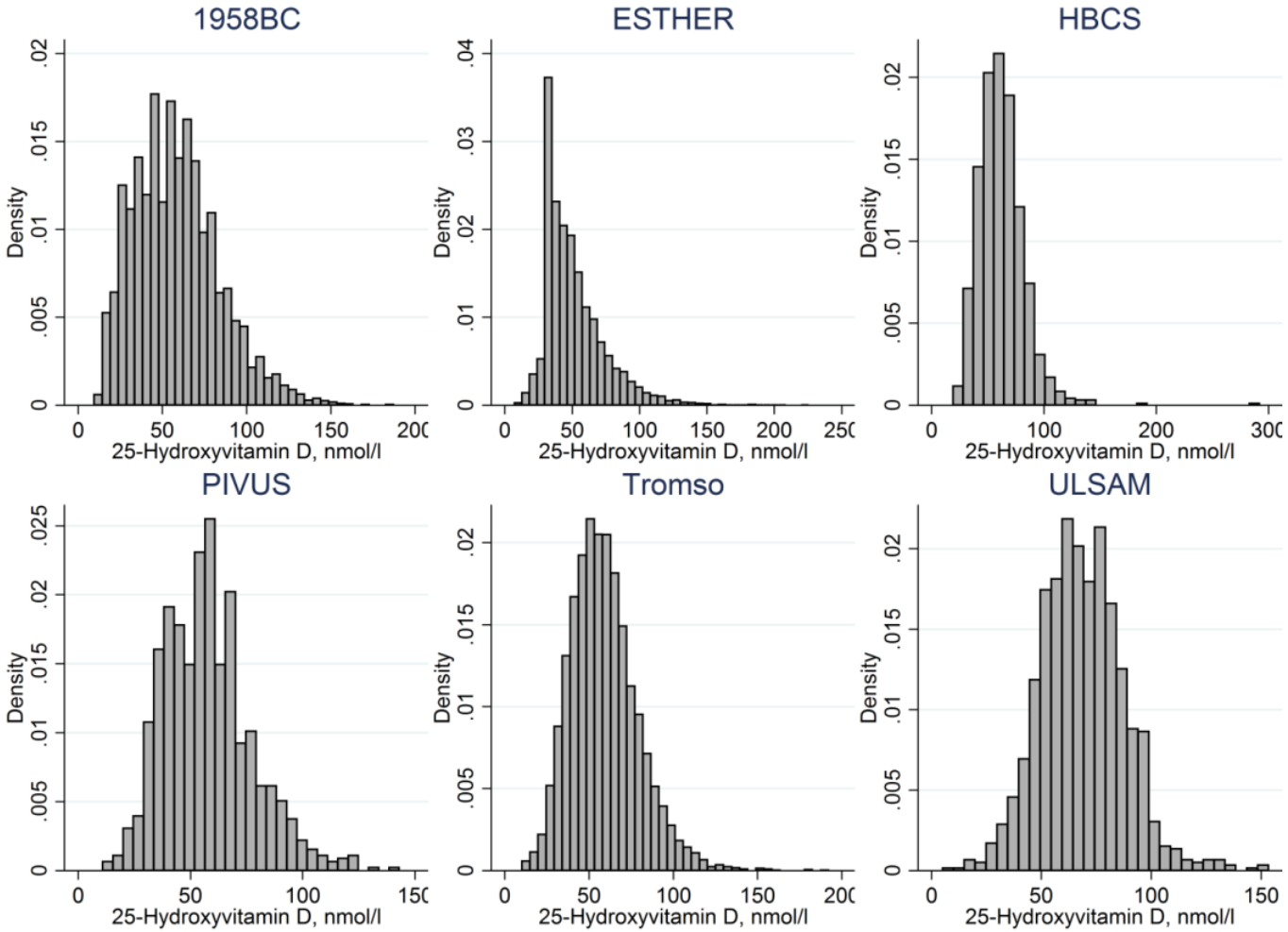
Appendix 6: Additional information for Chapter 8

Appendix 6.1: SNP quality checks

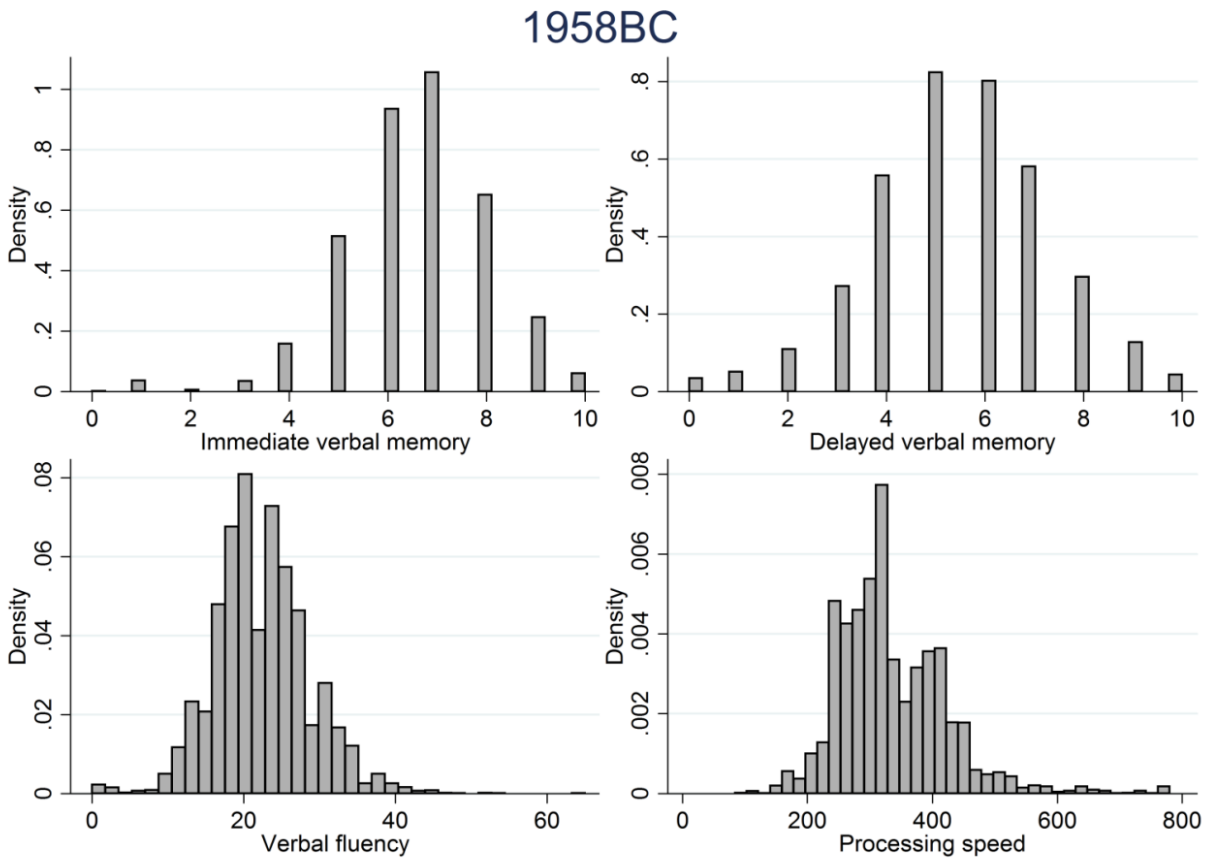
Study	SNP	Gene	Alleles Measured	Call Rate	MAF	HWE p-value	Imputed/Genotyped
1958BC	rs12785878	*DHCR7*	G/T	97.89	0.22	0.66	Genotyped
	rs12794714	*CYP2R1*	A/G	90.64	0.43	0.48	Genotyped
ASPS	rs12785878	*DHCR7*	G/T	100	0.29	0.11	Imputed from 1000G
	rs12794714	*CYP2R1*	A/G	100	0.44	0.72	Imputed from 1000G
ELSA	rs12785878	*DHCR7*	G/T	98.99	0.22	0.08	Genotyped
	rs12794714	*CYP2R1*	A/G	99.11	0.43	0.80	Genotyped
ESTHER	rs11603330	*DHCR7*	A/C	85.26	0.26	0.59	Genotyped
	rs12794714	*CYP2R1*	A/G	85.10	0.46	0.93	Genotyped
HBCS	rs7944926	*DHCR7*	A/G	89.95	0.27	0.38	Genotyped
	rs12794714	*CYP2R1*	G/A	90.01	0.39	0.64	Genotyped
PIVUS	rs12785878	*DHCR7*	C/A	97.62	0.35	0.29	Genotyped
	rs12794714	*CYP2R1*	G/A	89.21	0.40	0.78	Genotyped
TROMSØ	rs12785878	*DHCR7*	G/T	95.70	0.38	0.01	Genotyped
	rs12794714	*CYP2R1*	A/G	76.23	0.41	0.15	Genotyped
ULSAM	rs12785878	*DHCR7*	C/A	94.05	0.33	0.59	Genotyped
	rs7938266	*CYP2R1*	G/A	94.14	0.39	0.57	Genotyped
WHII	rs12785878	*DHCR7*	G/T	87.57	0.23	0.50	Genotyped
	rs12794714	*CYP2R1*	A/G	87.40	0.42	0.46	Genotyped

G: Guanine; T: Thymine; A: Adenine; C: Cytosine; MAF: Minor allele frequency; HWE: Hardy-Weinberg equilibrium

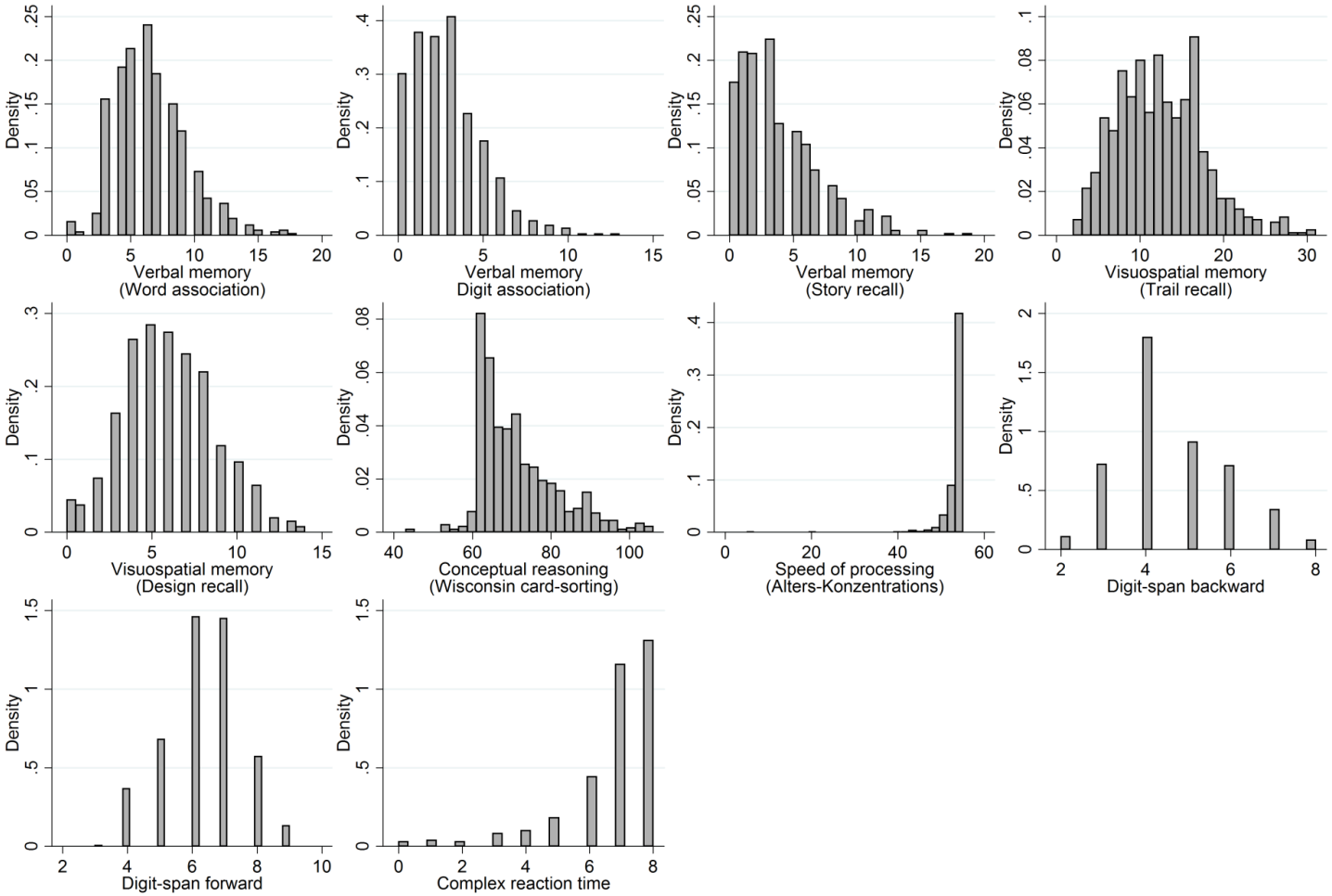
Appendix 6.2: Distribution of 25(OH)D in each cohort

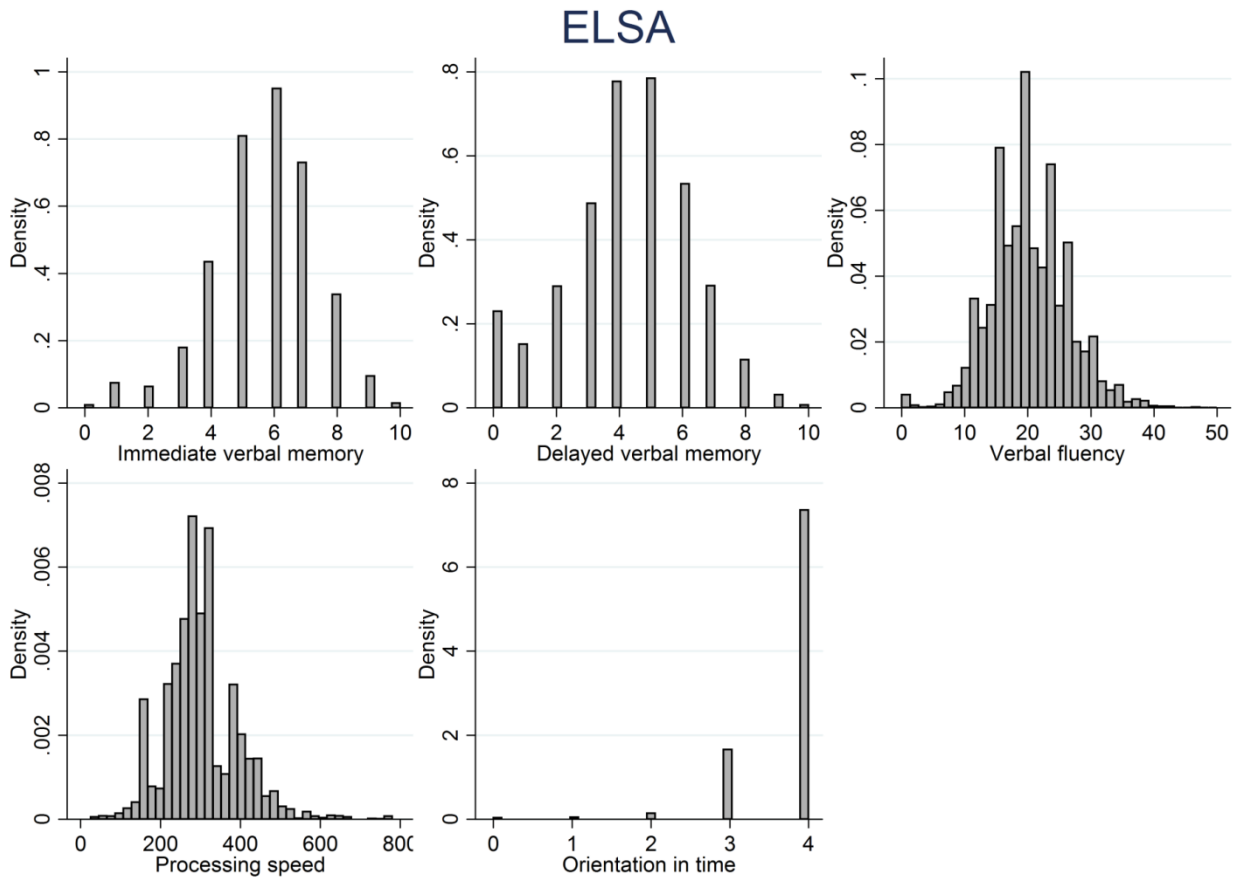


Appendix 6.3: Distribution of cognitive tests in each cohort

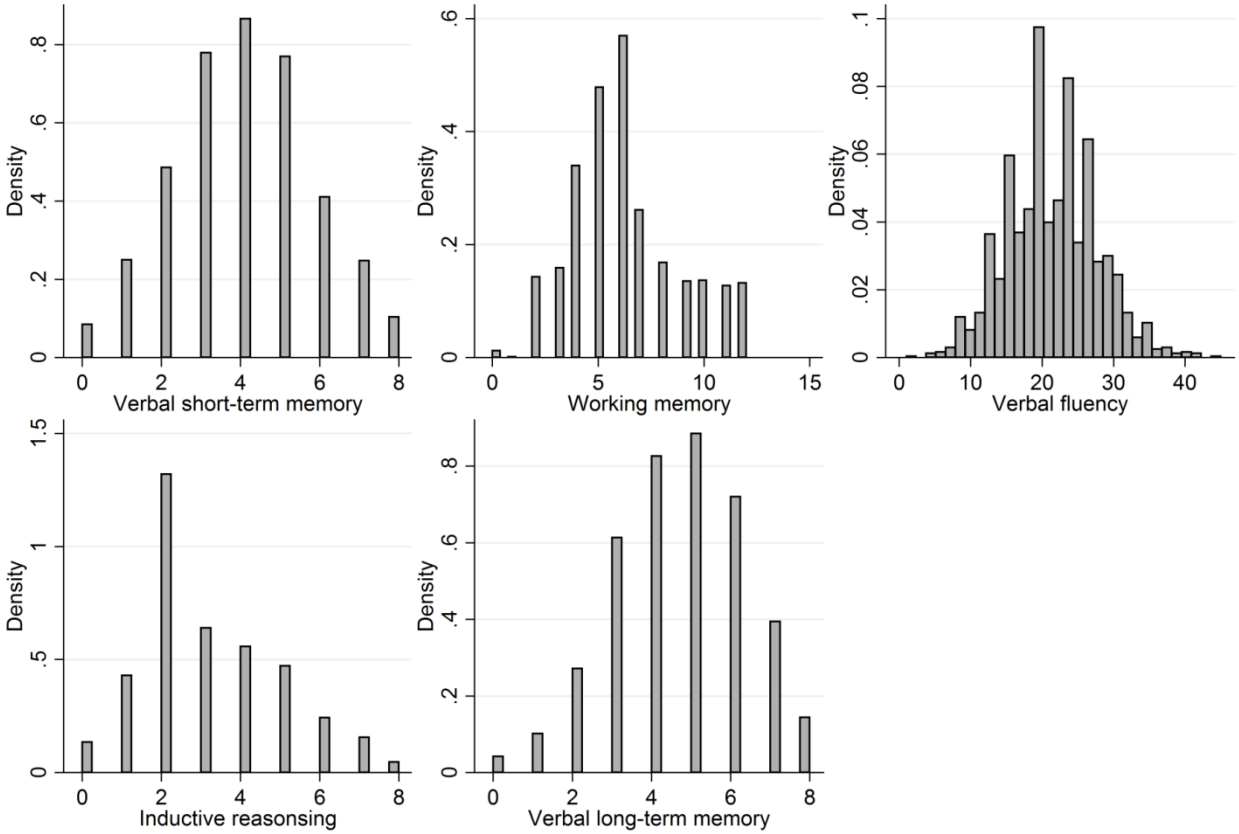


ASPS

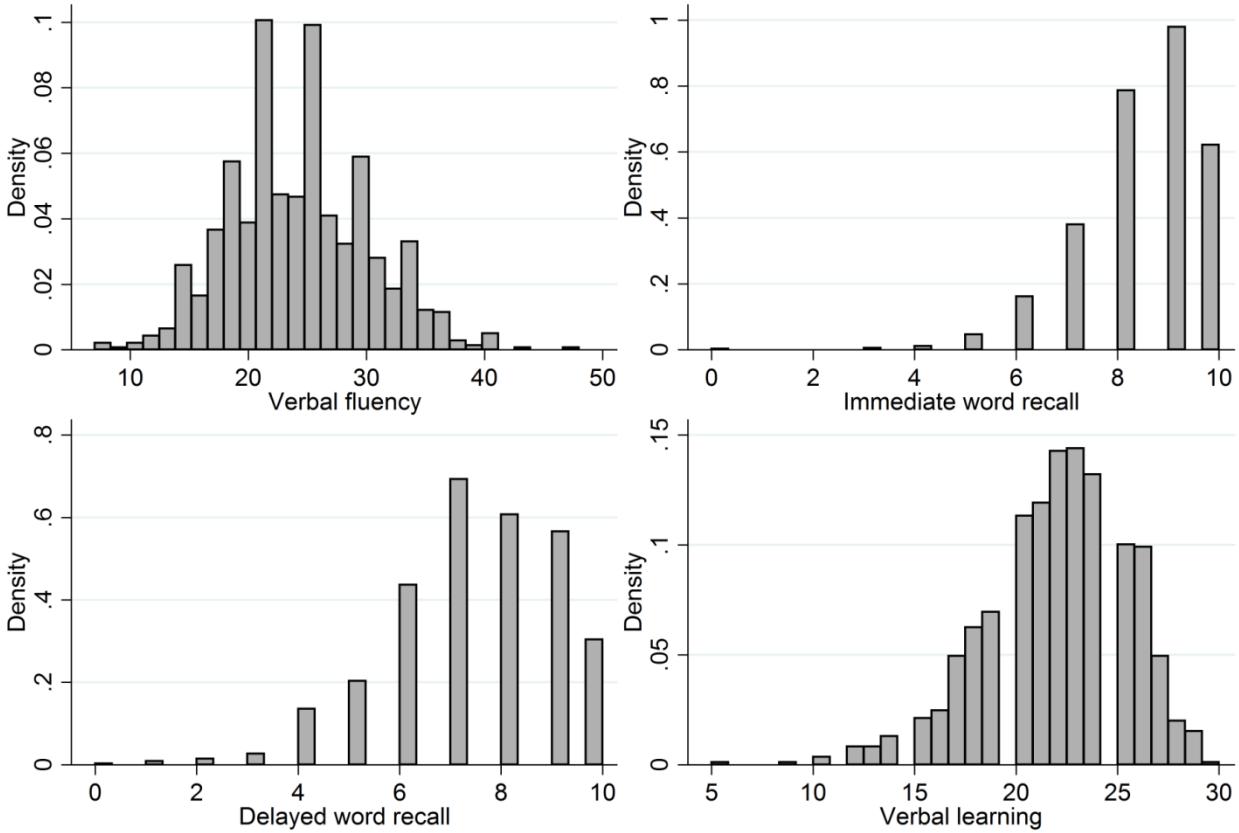

Density
Verbal memory
(Word association)
Verbal memory
Digit association)
Verbal memory
(Story recall)
Visuospatial memory
(Trail recall)
Visuospatial memory
(Design recall)
Conceptual reasoning
(Wisconsin card-sorting)
Speed of processing
(Alters-Konzentrations)
Digit-span backward
Digit-span forward
Complex reaction time

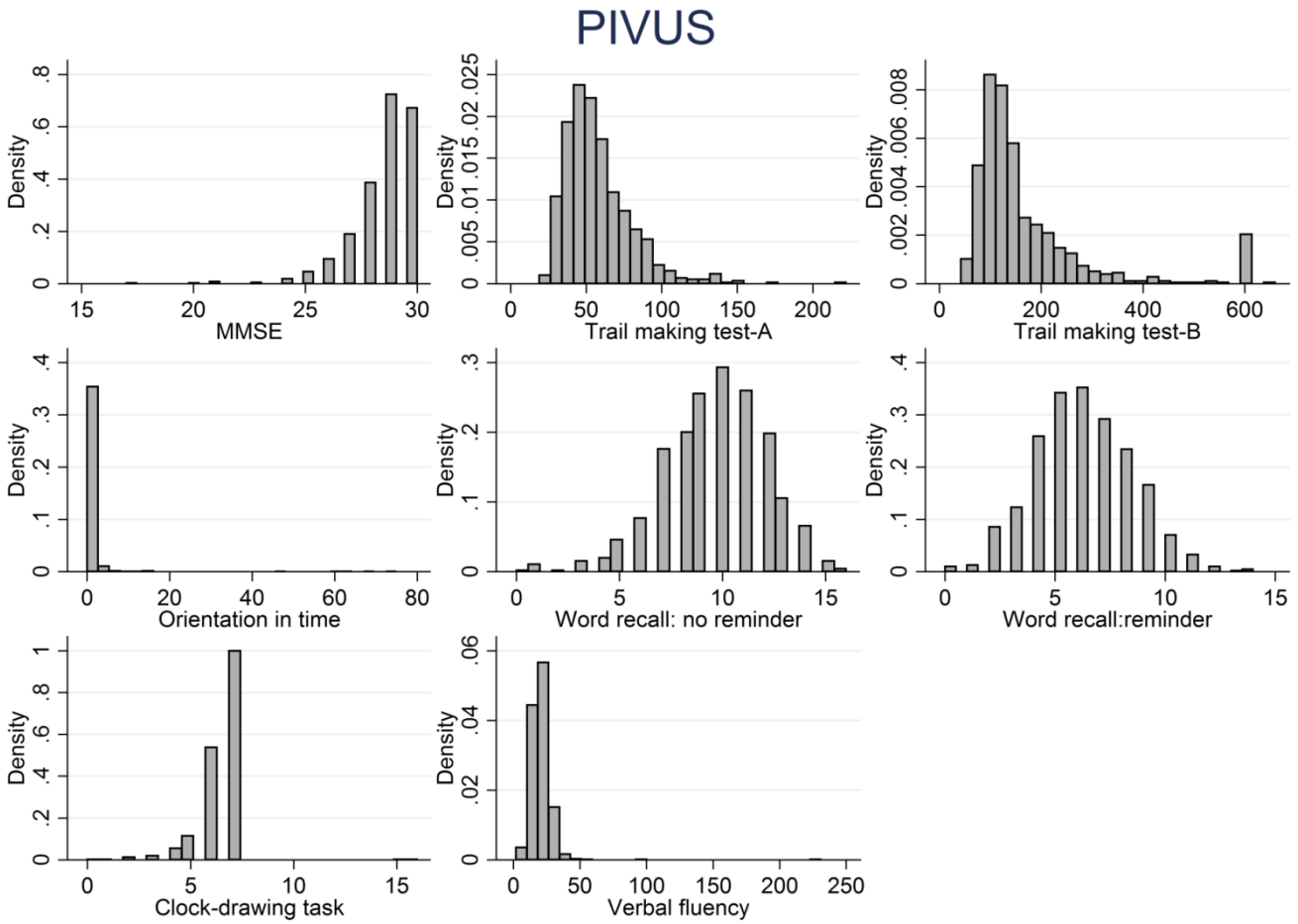

ELSA
Density
Immediate verbal memory
Delayed verbal memory
Verbal fluency
Processing speed
Orientation in time

ESTHER

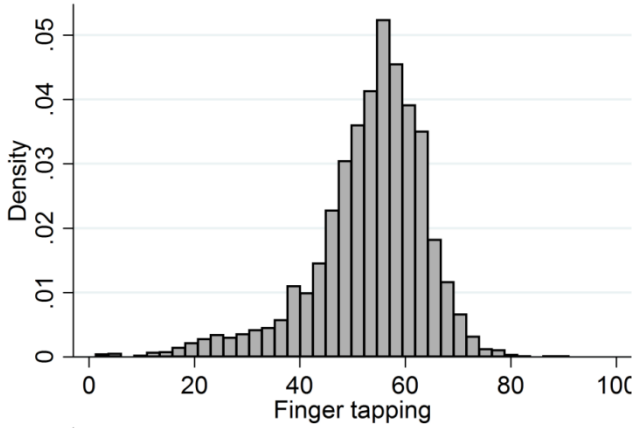

Density
0
.2
.4
.6
.8
0
2
4
6
8
Verbal short-term memory
Density
0
.2
.4
.6
0
5
10
15
Working memory
Density
0
.02
.04
.06
.08
.1
0
10
20
30
40
Verbal fluency
Density
0
.5
1
1.5
0
2
4
6
8
Inductive reasonsing
Density
0
.2
.4
.6
.8
0
2
4
6
8
Verbal long-term memory

HBCS


Density
0
.02
.04
.06
.08
.1
10
20
30
40
50
Verbal fluency
Density
0
.2
.4
.6
.8
1
0
2
4
6
8
10
Immediate word recall
Density
0
.2
.4
.6
.8
0
2
4
6
8
10
Delayed word recall
Density
0
.05
.1
.15
5
10
15
20
25
30
Verbal learning


PIVUS
Density
MMSE
Trail making test-A
Trail making test-B
Orientation in time
Word recall: no reminder
Word recall:reminder
Clock-drawing task
Verbal fluency

Tromso


Density
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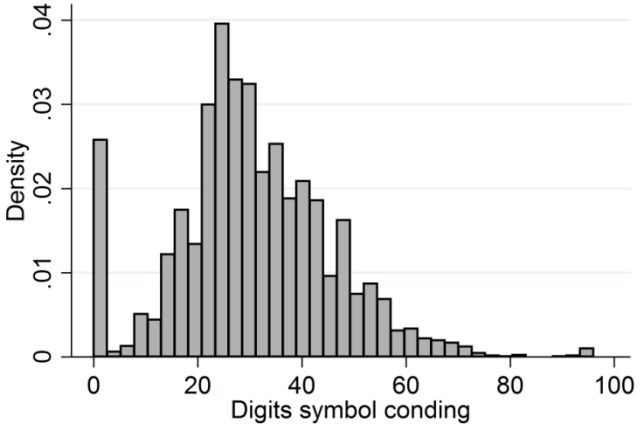
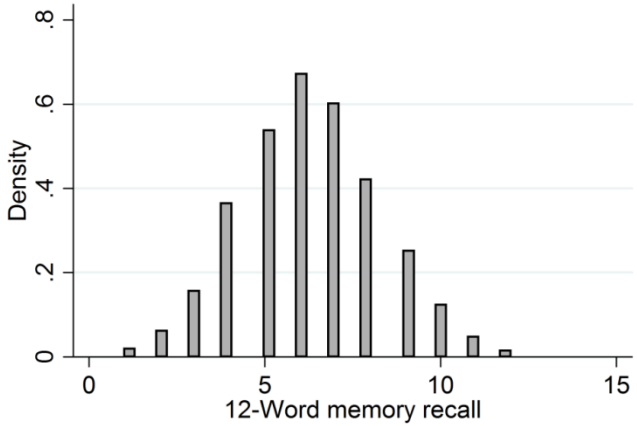
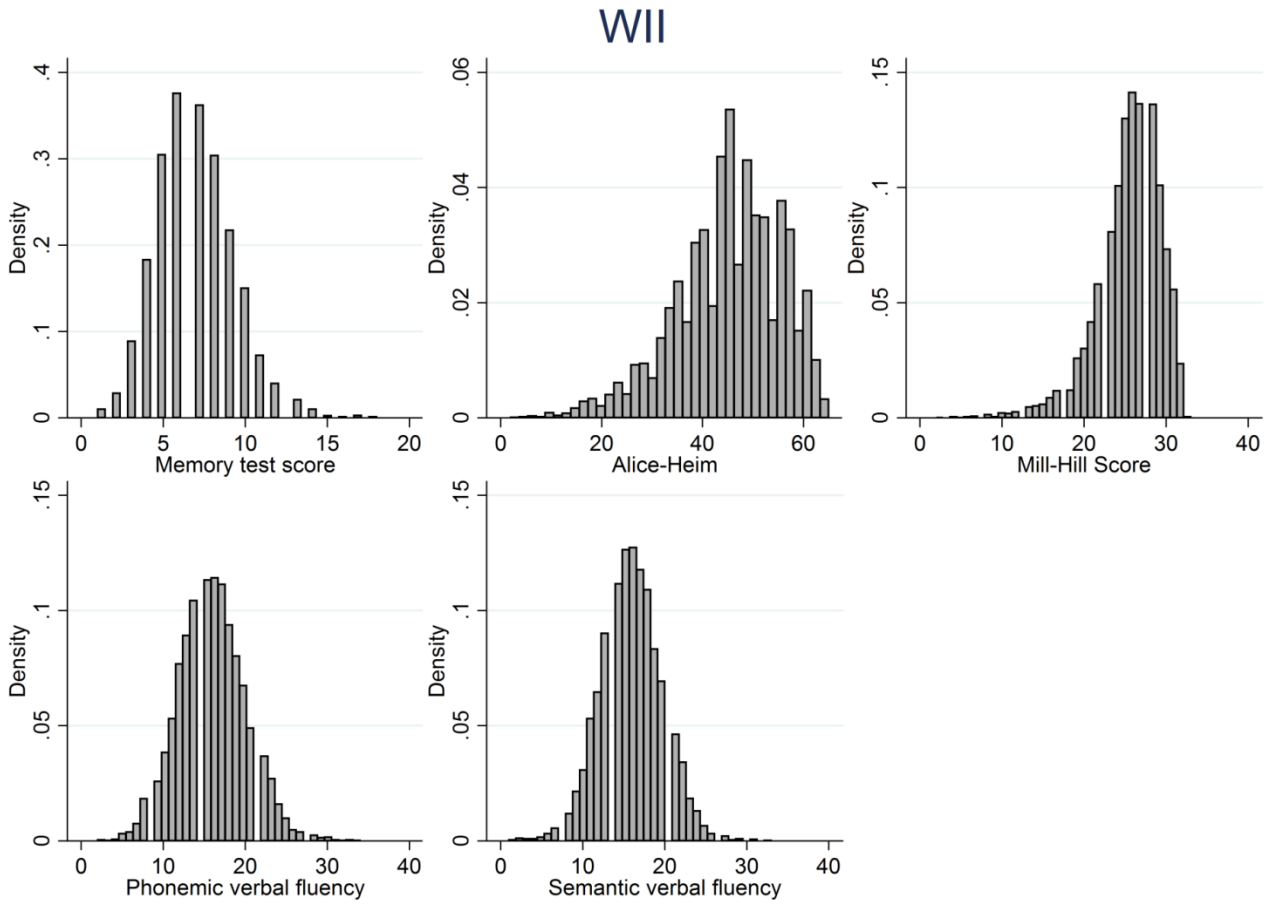
.02
.03
.04
.05
0
20
40
60
80
100
Finger tapping


Density
0
.01
.02
.03
.04
0
20
40
60
80
100
Digits symbol conding

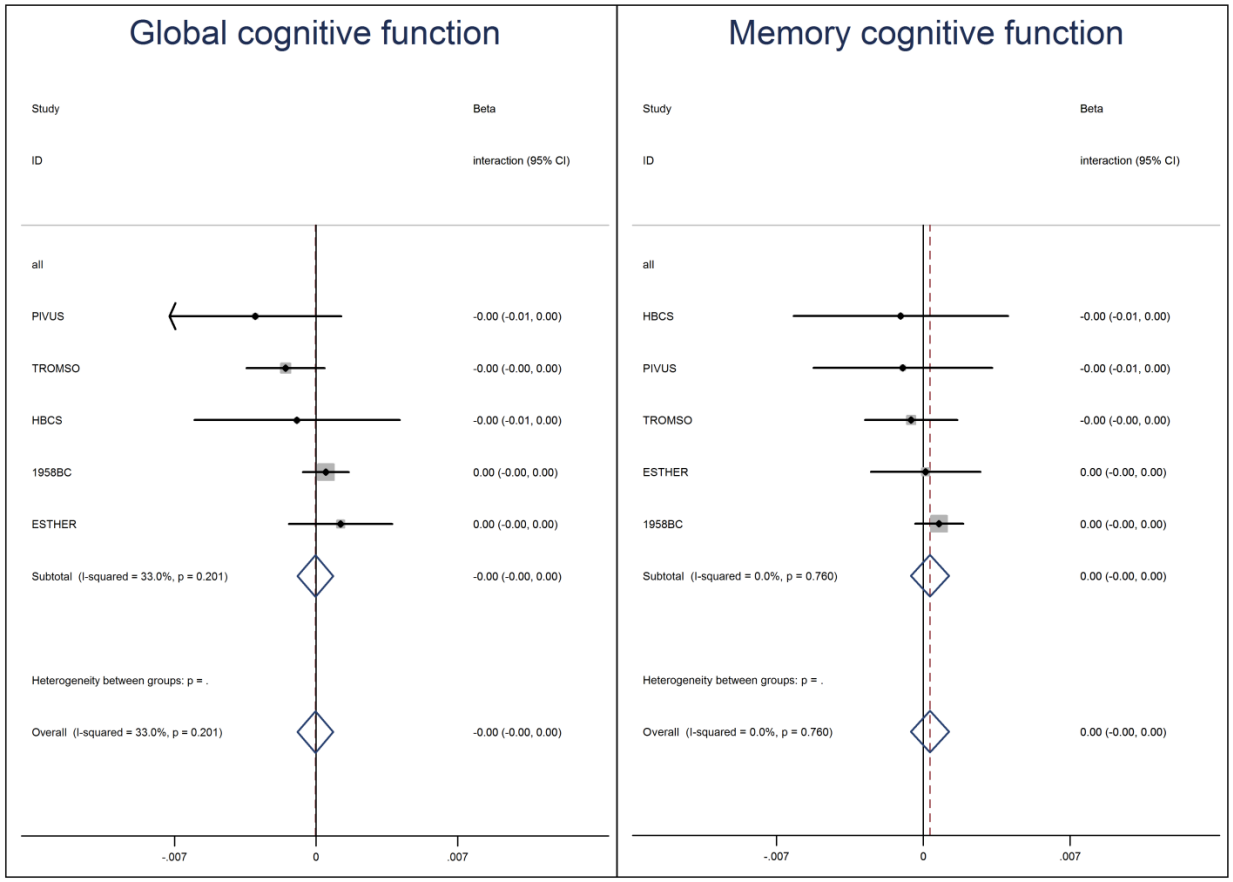

Density
0
.2
.4
.6
.8
0
5
10
15
12-Word memory recall


WII
Density
Memory test score
Alice-Heim
Mill-Hill Score
Phonemic verbal fluency
Semantic verbal fluency

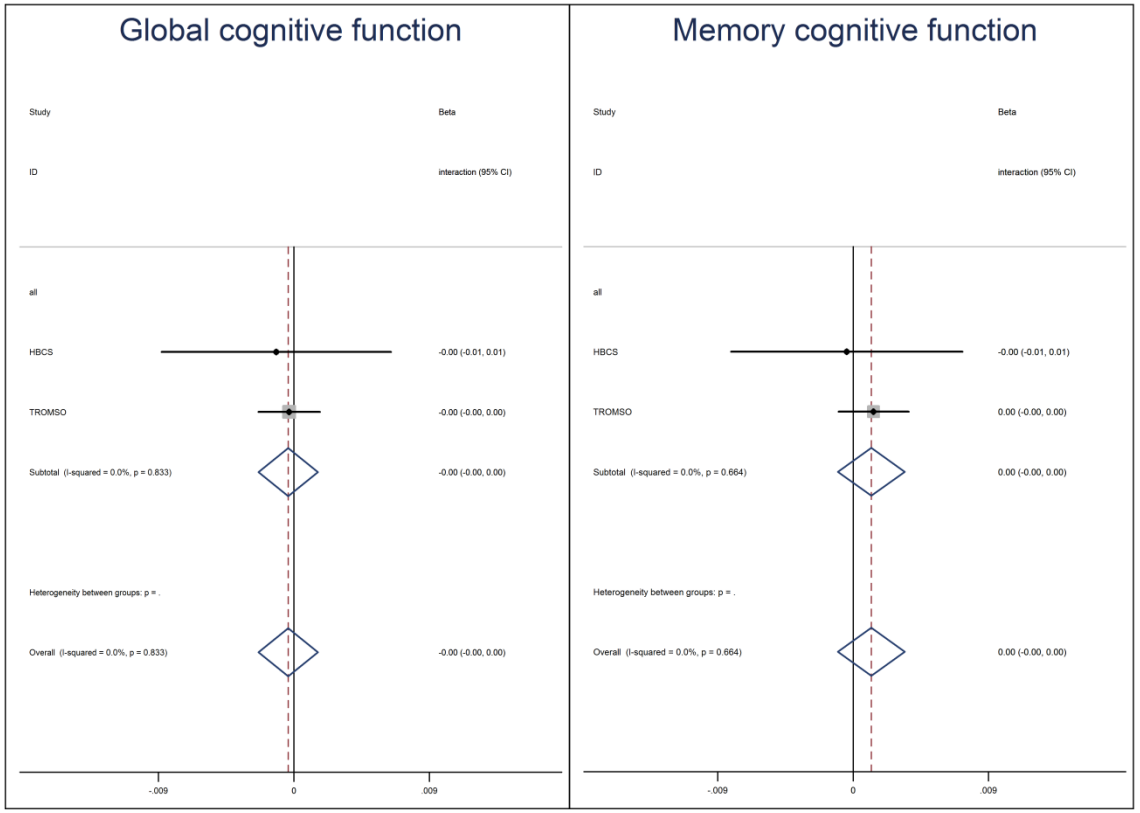
Appendix 6.4: Distribution of genetic variants in each cohort

	***DHCR7* per allele, %(N)**			***CYP2R1* per allele, %(N)**		
	0	1	2	0	1	2
1958BC	60.7 (3,770)	34.6 (2,147)	4.8 (295)	32.2 (1,853)	49.5 (2,847)	18.3 (1,052)
ASPS	48.56 (401)	44.0 (363)	7.5 (62)	31.1 (257)	50.0 (413)	18.9 (156)
ELSA	60.8 (3,328)	33.8 (1,853)	5.4 (295)	32.4 (1,776)	48.9 (2,681)	18.7(1,026)
ESTHER	55.0 (4,573)	38.5 (3,198)	6.5 (541)	29.0 (2,402)	49.8 (4,128)	21.3(1,766)
HBCS	37.6 (613)	48.5 (790)	13.9 (226)	37.4 (609)	48.1 (783)	14.6 (238)
PIVUS	43.3 (427)	43.8 (432)	12.9 (127)	36.4 (328)	47.5 (428)	16.1 (145)
TROMSO	38.6 (4,555)	46.1 (5,439)	15.4 (1,812)	34.5 (3,246)	49.1 (4,616)	16.4 (1,542)
ULSAM	44.8 (503)	43.7 (491)	11.5 (129)	36.2 (407)	48.7 (547)	15.1 (170)
WHII	59.9 (2,700)	34.8 (1,569)	5.3 (241)	33.4 (1,504)	48.3 (2,175)	18.3 (822)

Appendix 6.5: Effect modification by gender on 25(OH)D and cognitive function



Appendix 6.6: Effect modification by age-group (<65 versus ≥65 years) on 25(OH)D and cognitive function



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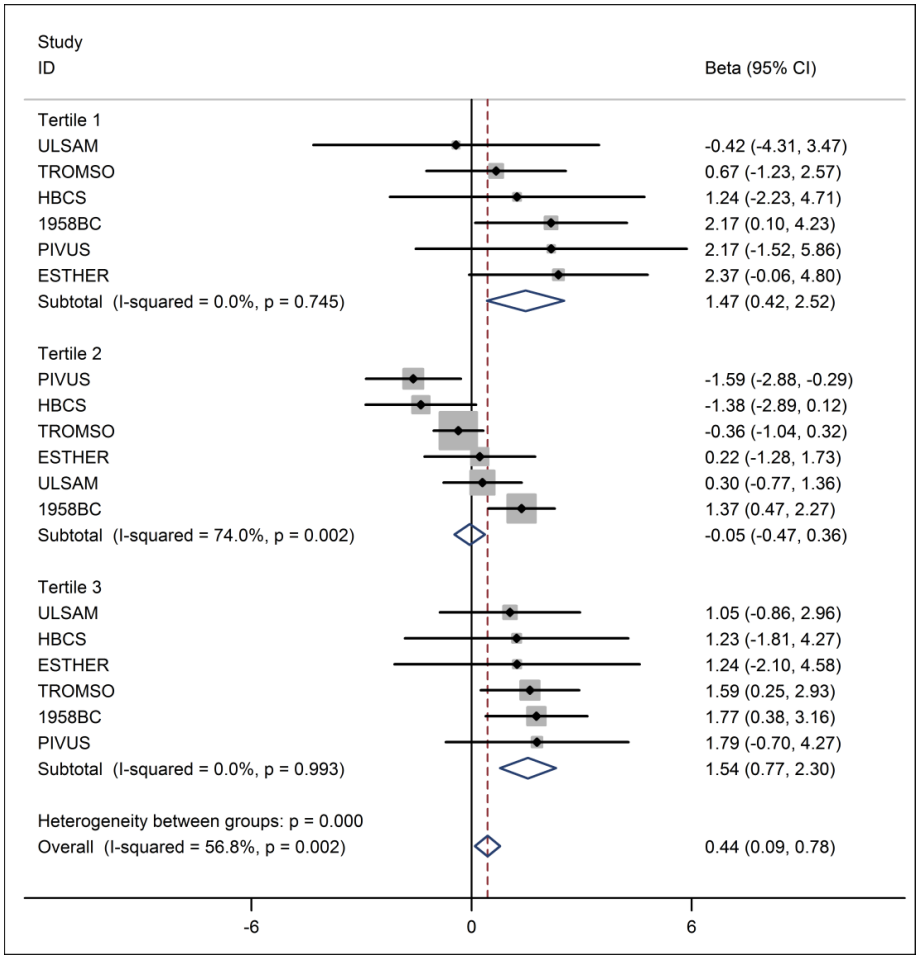
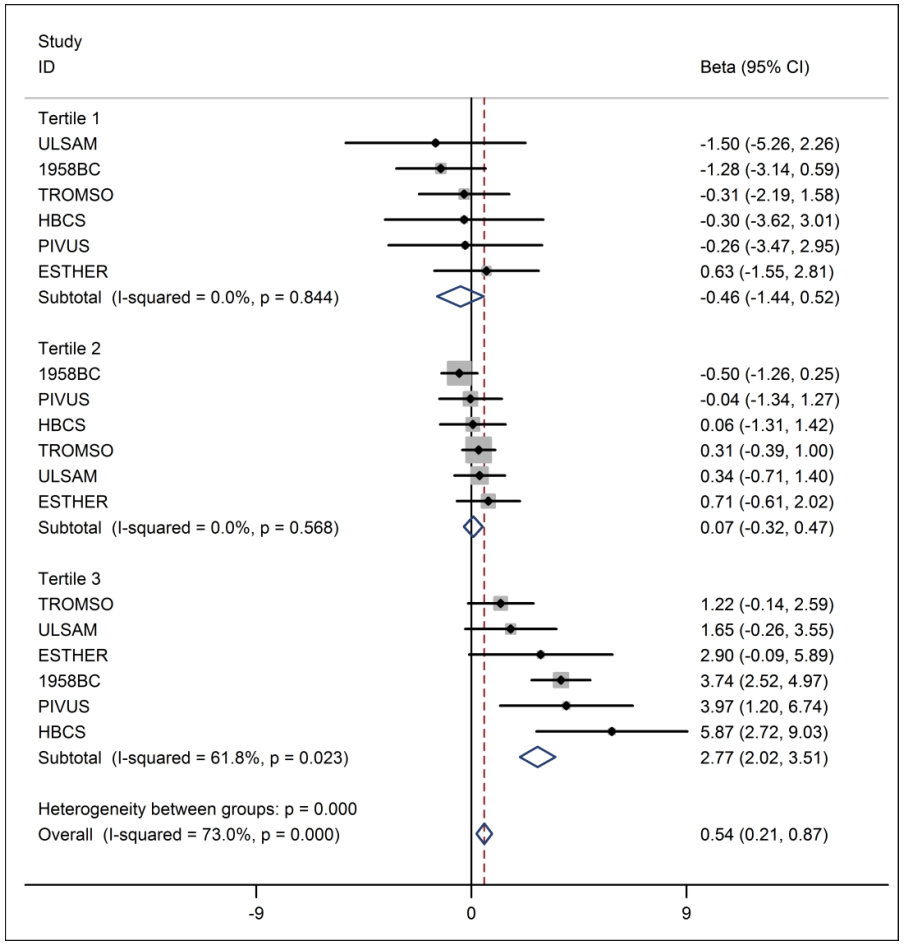
Appendix 6.7: Association between *DHCR7* and 25(OH)D, stratified by 25(OH)D tertiles.

Beta reflects % change in 25(OH)D per vitamin D-increasing allele



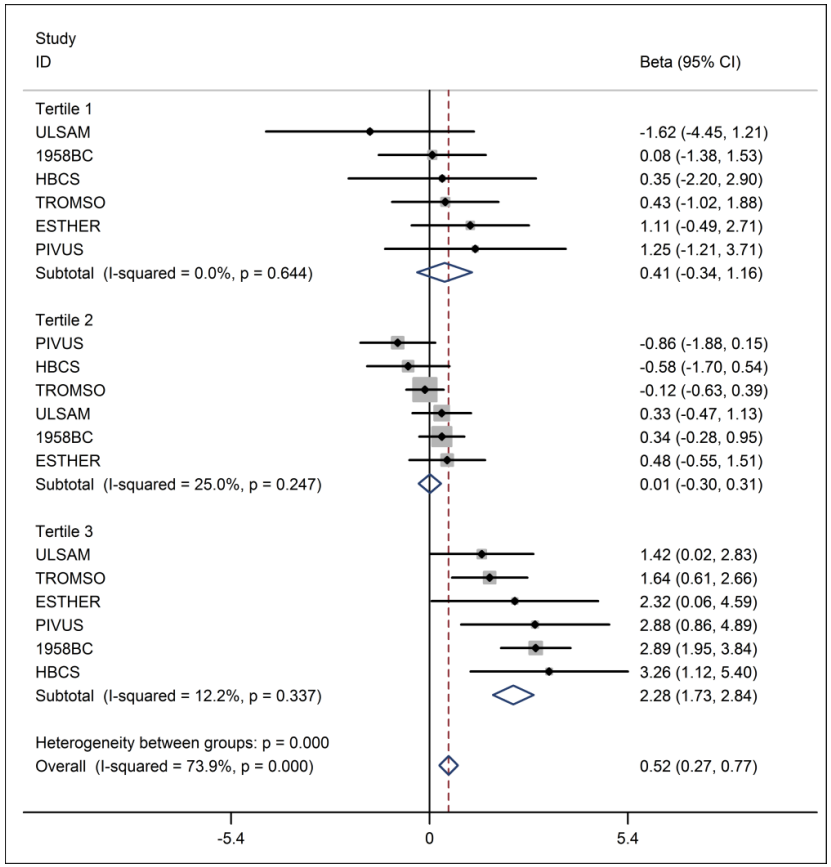
Appendix 6.8: Association between *CYP2R1* and naturally log-transformed 25(OH)D, stratified by 25(OH)D tertiles.

Beta reflects % change in 25(OH)D per vitamin D-increasing allele



Appendix 6.9: Association between synthesis score and naturally log-transformed 25(OH)D, stratified by 25(OH)D tertiles.

Beta reflects % change in 25(OH)D per vitamin D-increasing allele



Appendix 6.10: Association between genetic variants and cognitive function

Cohort	Outcome	Exposure	N	Coefficient	(se)	p-value
1958BC	Global cognitive function	*DHCR7*	5,304	-0.001	(0.02)	0.97
		CYP2R1	4,900	0.01	(0.02)	0.67
		Synthesis score	4,808	0.001	(0.02)	0.97
	Memory cognitive function	*DHCR7*	5,402	-0.0001	(0.02)	0.10
		CYP2R1	4,995	0.03	(0.02)	0.12
		Synthesis score	4,901	0.02	(0.02)	0.31
ASPS	Global cognitive function	*DHCR7*	724	0.08	(0.05)	0.09
		CYP2R1	724	0.04	(0.05)	0.40
		Synthesis score	724	0.06	(0.04)	0.07
	Memory cognitive function	*DHCR7*	763	0.08	(0.05)	0.08
		CYP2R1	763	0.03	(0.04)	0.57
		Synthesis score	763	0.05	(0.03)	0.11
ELSA	Global cognitive function	*DHCR7*	5,309	0.04	(0.02)	0.06
		CYP2R1	5,315	-0.03	(0.02)	0.12
		Synthesis score	5,270	0.002	(0.01)	0.87
	Memory cognitive function	*DHCR7*	5,421	0.03	(0.02)	0.23
		CYP2R1	5,429	-0.04	(0.02)	0.05
		Synthesis score	5,382	-0.01	(0.01)	0.50
ESTHER	Global cognitive function	*DHCR7*	1,338	0.06	(0.04)	0.15
		CYP2R1	1,339	0.03	(0.04)	0.46
		Synthesis score	1,331	0.04	(0.03)	0.13
	Memory cognitive function	*DHCR7*	1,338	0.04	(0.04)	0.39
		CYP2R1	1,339	0.02	(0.04)	0.58
		Synthesis score	1,331	0.03	(0.03)	0.30
HBCS	Global cognitive function	*DHCR7*	785	0.01	(0.05)	0.89
		CYP2R1	786	-0.04	(0.05)	0.41
		Synthesis score	785	-0.01	(0.04)	0.73
	Memory cognitive function	*DHCR7*	785	0.04	(0.05)	0.45
		CYP2R1	786	-0.04	(0.05)	0.41

Cohort	Outcome	Exposure	N	Coefficient	(se)	p-value
		Synthesis score	785	0.002	(0.04)	0.96
PIVUS	Global cognitive function	*DHCR7*	743	-0.02	(0.05)	0.75
		CYP2R1	684	-0.02	(0.06)	0.76
		Synthesis score	676	-0.02	(0.04)	0.72
	Memory cognitive function	*DHCR7*	768	0.02	(0.05)	0.78
		CYP2R1	705	-0.003	(0.06)	0.96
		Synthesis score	697	0.01	(0.04)	0.73
Tromsø	Global cognitive function	*DHCR7*	3,535	-0.0004	(0.02)	0.98
		CYP2R1	2,275	-0.05	(0.02)	0.02
		Synthesis score	2,253	-0.04	(0.02)	0.01
	Memory cognitive function	*DHCR7*	3,755	0.03	(0.02)	0.09
		CYP2R1	2,407	-0.05	(0.03)	0.04
		Synthesis score	2,385	-0.03	(0.02)	0.15
ULSAM	Global cognitive function	*DHCR7*	833	0.04	(0.05)	0.48
		CYP2R1	832	-0.09	(0.05)	0.07
		Synthesis score	829	-0.03	(0.04)	0.44
WHII	Global cognitive function	*DHCR7*	4,040	-0.01	(0.02)	0.64
		CYP2R1	4,038	-0.002	(0.02)	0.91
		Synthesis score	3,961	-0.01	(0.02)	0.58
	Memory cognitive function	*DHCR7*	4,062	-0.01	(0.03)	0.82
		CYP2R1	4,060	-0.01	(0.02)	0.79
		Synthesis score	3,983	-0.01	(0.02)	0.58

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3. Lawlor DA, Harbord RM, Sterne JAC, et al. (2008) Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. *Stat Med*. **27**(8):1133-63.

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